Determination of Carbonic Acid Species Using Carbonate- and Novel Bicarbonate-Selective Electrodes

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Prologue

Over the course of time, Åbo has become a second home to me. Even though the time spent here was brief, the people, the university, and the city itself have painted a familiar picture. In Åbo, there was warmth even during the cold dreadful winters and friendship to be found in every corner. There's a lot to be thankful for and this space is dedicated to those who made it possible for me to come this far.

From Sri Lanka, to United States, to Estonia, to Malta, to Finland, has been an extraordinary journey. Thank you, Ivo, for being the master mind behind the EACH program and creating a wonderful experience for students around the world. Thank you, Johan, for opening up Åbo Akademi University for us and making us feel welcome.

Thank you Tomek, Zekra, Kim, and Johan not only for being my supervisors here at Åbo but also for your continuous support, encouragement, and guidance. Also, thank you, Tom, Rose-Marie, and Sten for being a constant presence and making our stay even more wonderful. Thank you, Prof. Jonas Bergquist and Prof. Jérôme Randon, for two amazing winter schools in Sweden and France.

Last but not least, thank you my colleagues, Slim and Jaypee, for stimulating discussions but most importantly for continuous laughter we shared, without which the lab work would have been mundane.

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List of Abbreviations Used in this Thesis

ISE	Ion-Selective Electrode
ISM	Ion-Selective Membrane
Eqn.	Equation
CWE	Coated Wire Electrode
SCISE	Solid-Contact Ion-Selective Electrode
SPISE	Single-Piece Ion-Selective Electrode
CPBISE	Conducting-Polymer-Based Ion-Selective Electrode
THF	Tetrahydrofuran
PEDOT	Poly(3,4-ethylenedioxythiophene)
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
EMF	Electromotive Force
SSM	Separate Solution Method
MSM	Mixed Solution Method
FPM	Fixed Primary Ion Method
FIM	Fixed Interfering Ion Method
DIC	Dissolved Inorganic Carbon

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Chapter 1: Introduction

In human physiology, bicarbonate ions play a vital role. The pH level in blood is strictly regulated between 7.35 and 7.45 pH units [1]. There are three primary systems within the human body that regulates the hydrogen ion concentration, thus maintaining the acid-base balance. These three systems operate in different time scales. First, the chemical buffer systems in body fluids react immediately towards any sudden changes in the H^+ concentration. Nevertheless, the buffer systems do not remove nor add H^+ into the body. Instead, the ions are tied up until the acid-base equilibria can be restored. Secondly, the respiratory system, within a span of few minutes, eliminates CO_2 (therefore H_2CO_3) from the body. The final line of defense against acid-base disturbances are the kidneys. However, kidneys have a much slower response time. Therefore, the first two mechanisms are vital to maintain the balance until the kidneys can successfully eliminate excess amount of acid or base from the body [2].

The bicarbonate buffer system in body fluids is one of the most important buffer systems responsible for regulating the blood pH. Thus, a notable change in bicarbonate levels in blood may indicate metabolic acidosis or metabolic alkalosis, although this information needs to be correlated with other biochemical parameters for an accurate diagnosis. Nonetheless, bicarbonate values in biological fluids are of critical importance in diagnosis and management of a variety of emergencies and chronic diseases, particularly pulmonary and renal diseases [2]. Even though this is a critical parameter, a reliable ion sensor for direct determination of bicarbonate levels in biological fluids has not yet been invented [3].

Perhaps it is worth turning the pages of history, albeit briefly, to gain a comprehensive understanding of the importance of blood gas analysis which consequently led to studies on acid-base equilibria. Mere twenty years after the invention of the vacuum pump, Robert Boyle and Robert Hook obtained air from blood using a special vacuum pump created by Boyle in 1670 and yet the credits for the discovery of carbon dioxide belongs to Joseph Black which he first termed as “fixed air” in 1754 [4]. Three scientists are primarily associated with the discovery of oxygen. Swedish chemist Carl Wilhelm Scheele first discovered oxygen (which he called “fire air”) in 1772 although this discovery was not published until 1777 [5]. Therefore, Joseph Priestley is often credited for the discovery of oxygen (called “dephlogisticated air”) in 1774 [6]. Finally, it was Antoine Lavoisier who coined the term “oxygen” [5]. Despite these discoveries, the first reliable blood gas analysis was performed by Gustav Magnus in 1837

although his estimates for oxygen, carbon dioxide, and nitrogen were much lower than the nominal values that are known today [4].

The pioneer of the modern era of blood gas analysis and acid-base equilibria is perhaps Donald Dexter Van Slyke who is also considered to be one of the founders of modern quantitative blood chemistry [7]. Van Slyke and John P. Peters co-authored the book "*Quantitative Clinical Chemistry: Volume I: Interpretations*" (1931) and the 18th chapter of this book is renowned as "*one of the most precise, lucid, and comprehensive accounts of acid-base equilibrium that has ever appeared in scientific literature*" [8, p. 191]. Van Slyke has published 317 articles [9] which includes significant contributions to a series of 23 papers titled "*Studies of Acidosis*" [10]. The 17th article of this series "*Studies of Acidosis: The Normal and Abnormal Variations in the Acid-Base Balance of the Blood*" (1921) was monumental in understanding acid-base balance pathology [11]. He further developed a method of acid-base chemistry relied upon determining the plasma CO_2 content utilizing his manometric apparatus (Manometric Van Slyke Apparatus) in 1924 which persisted as a standard method until the 1960s and was later replaced by the three-electrode method of blood gas analysis [12]. The Van Slyke methods have been described so extensively in his book "*Quantitative Clinical Chemistry: Volume II: Methods*" that an article published in the Journal of Research of the National Bureau of Standards (U.S. Department of Commerce) recommended it as a standard text [13]. Indeed, there are a multitude of chemists, physicists, physiologists etc., whose names are not mentioned here, that inspired Van Slyke's and others' research in this field. It is not the intention nor the scope of this thesis to do an all-inclusive historical review. However, it would be incomplete and folly without mentioning Lawrence Joseph Henderson and Karl Albert Hasselbach. Henderson's discovery of carbonic acid's ability to preserve neutrality in aqueous solutions, his derivation of Henderson equation (Eqn.1), and Hasselbach's adaptation of Henderson's equation and its rearrangement into the very well-known Henderson-Hasselbach equation (Eqn. 2) became iconic in all acid-base equilibria research that followed [14].

$$K = \frac{[H^+][HCO_3^-]}{[dCO_2]} \qquad \text{Eqn. 1}$$

Where dCO_2 includes both dissolved CO_2 gas and H_2CO_3.

$$pH = pK + \log\left\{\frac{[HCO_3^-]}{[dCO_2]}\right\} \qquad \text{Eqn. 2}$$

Apart from the volumetric, gasometric, and titrimetric methods for blood gas analysis developed by Van Slyke et al., Glenn E. Cullen also proposed a colorimetric method of

determining hydrogen ion concentration in blood plasma in 1922 [15]. Charles W. Boone and John B. Field devised a simple syringe titrator and colorimeter method for determination of serum bicarbonate in 1953 [16]. In 1955, Sidney Davis and T.H. Simpson Jr described a flame photometric method of measuring serum bicarbonate [17].

As mentioned before, one of the modern methods of blood gas analysis is dependent upon three electrodes; the pH electrode (Max Cremer, 1909), pCO_2 electrode (Richard Stow, 1954), and O_2 electrode (Leland Clark, 1954-1956) [14]. With the invention of these electrodes, the studies on acid-base equilibria have gradually shifted towards electrochemical methods. However, it is also important to note that there exist gas chromatographic methods of determining blood gases in biological fluids [18] and ion chromatographic method of determining bicarbonate in biological fluids [19].

The focus of this thesis is on carbonate and bicarbonate determination especially in biological fluids. Once the standard methods of determining carbonate and bicarbonate content in blood turned towards electrochemistry, there have been significant amount of research conducted in order to improve the accuracy of these methods. There are three components in a carbonate system; CO_2, CO_3^{2-}, and HCO_3^-. The dynamic equilibria between these three species are dependent upon the pH, which will be discussed in detail later in this thesis. In physiological pH, bicarbonate is the dominant form (figure 1). Since there exist no direct method of determining bicarbonate in biological fluids, the bicarbonate values are often presented as calculated values from pH, pCO_2, and CO_3^{2-}.

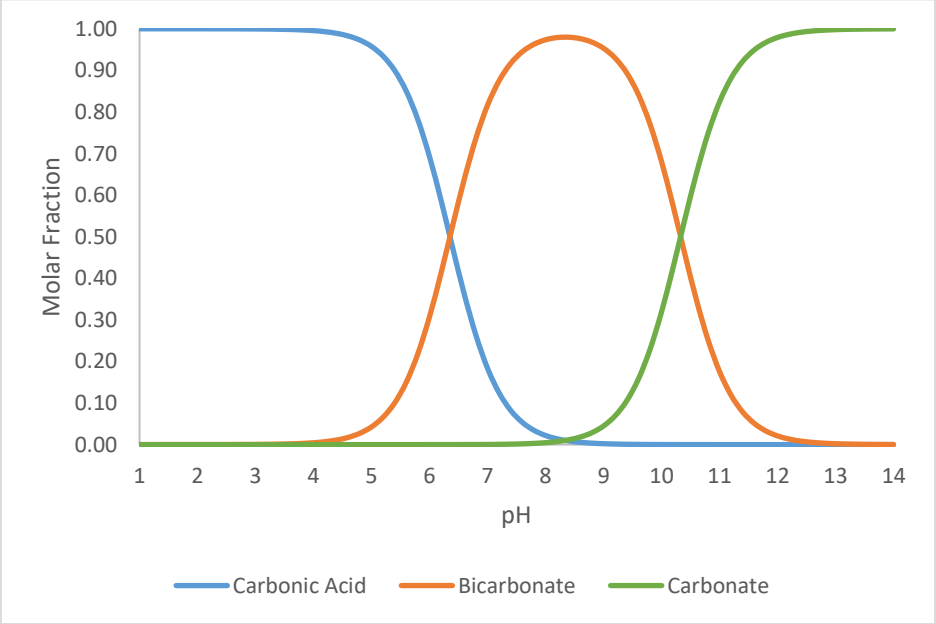


Figure 1: Dynamic Equilibrium Between Carbonic Acid Species in Varying pH

In 1973, a US patent was registered for “bicarbonate ion-selective electrodes” [20]. However, the following year it was proven by Herman and Rechnitz that this electrode is more selective towards carbonate than bicarbonate [21]. Nevertheless, this discovery inspired a great amount of research on carbonate selective electrodes utilizing trifluoroacetophenone and its derivatives as carbonate ionophores [22]. In 2003, Bobacka et al. described a carbonate selective electrode with reduced interference from salicylate [23]. A chronopotentiometric method for carbonate detection was also proposed in 2014 although this was not suitable for clinical analysis due to limited working pH range [24]. Very recently (2017), Lewenstam et al. have successfully measured bicarbonate levels in mineral waters with an ion-selective sensor utilizing quaternary ammonium salts as an ion-exchanger [25]. However, this electrode was not effective in determining bicarbonate levels in biological fluids either.

Since Gustav Magnus’s blood gas analysis up until now, the field of clinical analysis has grown significantly more sophisticated. Despite nearly two centuries of advances, it is very evident that there are still many challenges remaining. Especially anion-selective electrodes lag behind notwithstanding the development in cation selective electrodes. Therefore, the main goal of this thesis is to kickstart a pilot study on a novel ionophore (figure 2) selective towards bicarbonate ions.

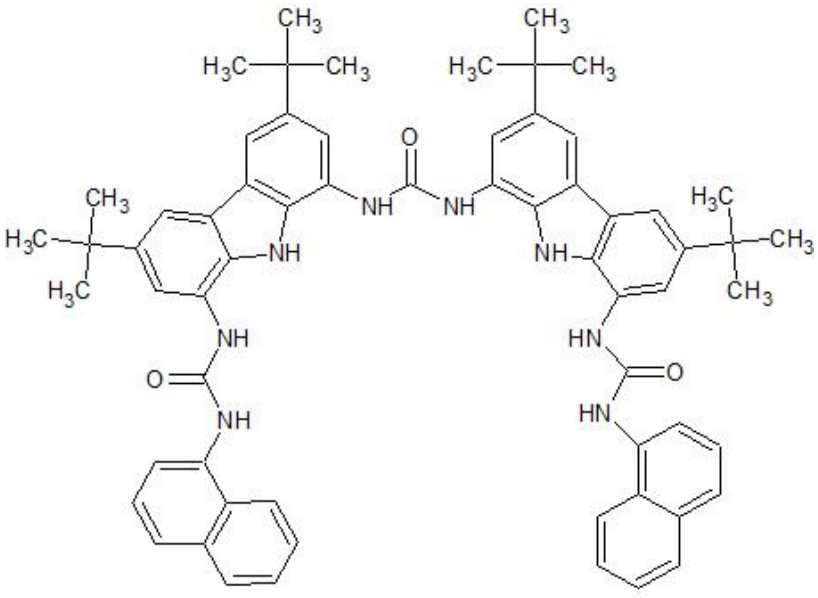


Figure 2: Bicarbonate Ionophore (Drawn with ChemSketch Freeware)

It is expected that this new ion-selective electrode would provide a direct method of determining bicarbonate levels in biological fluids. This ionophore has been synthesized at the Institute of Chemistry, University of Tartu, Estonia [26]. It has been observed that this

ionophore is almost equally selective towards both bicarbonate and acetate (manuscript under preparation). This thesis focuses the ionophore's selectivity towards bicarbonate ions.

1.1 Ion-Selective Electrodes

The history of Ion-Selective Electrodes (ISEs) can be traced all the way back to 1906 when Max Cremer discovered pH sensitive glass membranes [27]. In 1909, the pH electrode was developed and by 1936, the first commercial pH electrode was introduced in the United States [28]. Even though the pH electrode has evolved greatly over the last century, it still is the best-known ISE. While glass membrane electrodes dictate most of the pH measurements, the ionophore-based ISEs are used to quantify more than 70 different analytes including organic and inorganic ions, non-ionic species such as phenol derivatives, and non-ionic surfactants [29]. The purpose of an ISE is to respond to the activity of the target ion according to the Nernst Equation (Eqn. 9), which is discussed in detail in section 2.1 (potentiometry). The response of the ISE is the potential difference established at the Ion Selective Membrane (ISM) and the sample solution boundary. The primary component of an ISE is its ion selective membrane. In conventional ISEs (figure 3a), the ISM is positioned between the sample solution and a solution with a constant activity (the inner filling solution). An internal reference electrode which acts as the ion-to-electron transducer completes the circuit [30]. In an ISE, all potentials contributing to the measured potential, except the membrane | sample interface potential are kept constant. Thus, the measured potential difference can be attributed to the activity of the analyte.

While ISEs have multiple advantages over other systems, such as portability, simplicity, miniaturization possibility, low cost, and low energy consumption [31], the conventional ISEs do come with their own set of drawbacks. The major disadvantage of the conventional ISEs is the inner filling solution. Maintaining the level of the inner filling solution can be tedious. This also means that it leaves little room for miniaturization of the electrode since it risks evaporation of the solution rapidly. Furthermore, the electrode can only be used in a vertical position because the inner filling solution must be in contact with the ion selective membrane surface. The construction of the conventional electrode is also fragile, which limits its uses to mild laboratory and environmental conditions.

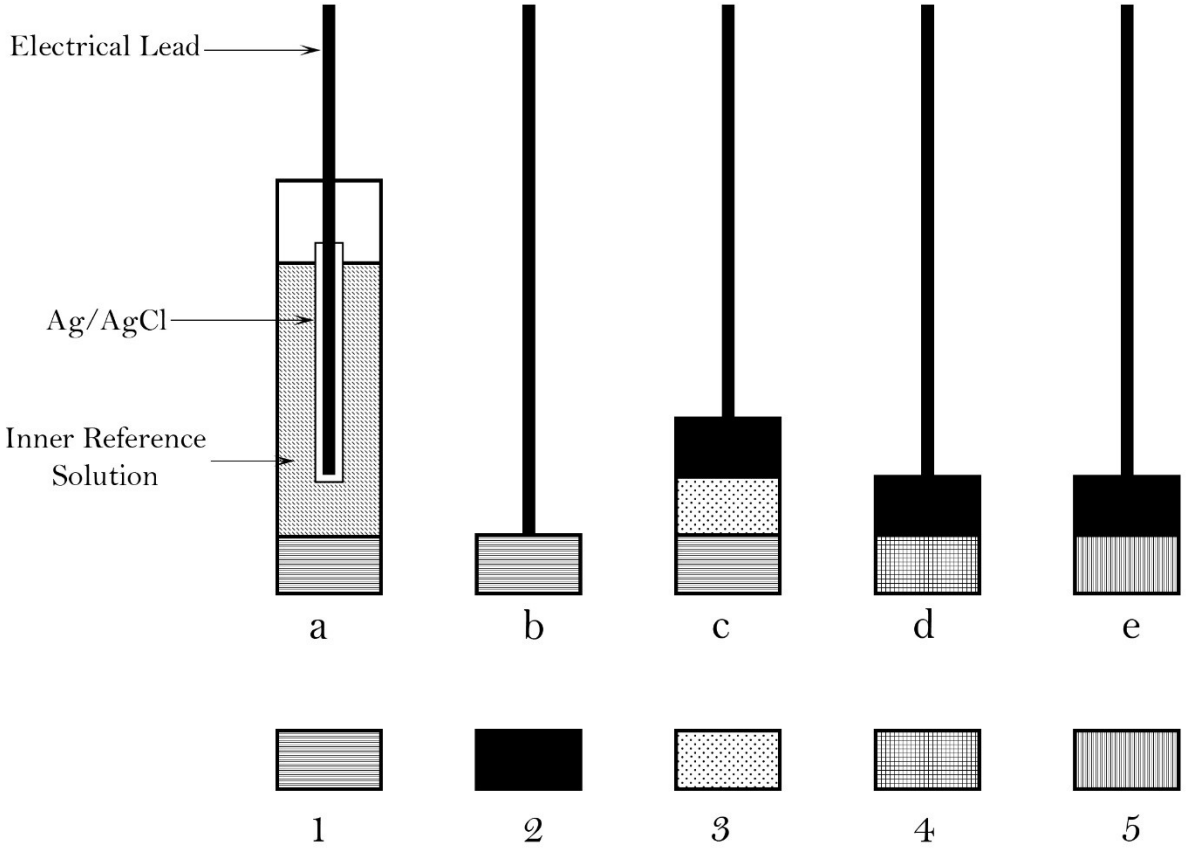


Figure 3: Schematic structure of a) Conventional ISE, b) CWE, c) SCISE, d) SPISE, and e) CPBISE. And 1) ISM, 2) Electronic conductor, 3) Conducting polymer, 4) Conducting polymer + ISM, and 5) Conducting polymer containing ion-recognition sites

In order to combat with the said disadvantages, a new type of ISE was needed. In 1971, Cattrall and Freiser invented the Coated Wire Electrode (CWE) (figure 3b) [32]. Very simple in design and construction, this was essentially a conducting wire (platinum, silver, glassy carbon, copper etc.) coated with an ion selective membrane. The CWE has several advantages over the conventional electrodes. Namely, lower cost, flexibility, simplicity, and miniaturization potential [33]. However, the CWE was not ideal either. Since there is no ion-to-electron transducer included in the design, there is a high charge transfer resistance at the interface between the electronically conducting wire and the ionically conducting membrane, which resulted in potential drifts and irreproducible readings [34].

The apparent solution to the shortcomings seen in the CWE is to include a solid ion-to-electron transducer in the design. This was achieved by employing a conducting polymer, which was invented in 1977, between the electronically conducting substrate and the ionically conducting membrane [35]. The conducting polymers are discussed in detail in section 1.2 (Conducting Polymers). These solid-contact ion-selective electrodes (SCISE) (figure 3c) have shown promising results and advanced significantly over the years.

Furthermore, two more types of all-solid-state ISEs have been developed. A single-piece ion-selective electrode (SPISE) (figure 3d) which has the conducting polymer integrated in the ISM. In such electrodes, the conducting polymer should be soluble in the same solvent used to dissolve the membrane components. While the conducting polymer still acts as the ion-to-electron transducer here, it may also play a role in selectivity since it is integrated in the membrane itself. Here lies the fundamental difference between SCISE and SPISE. The other type of solid-state ISE is the conducting-polymer-based ion-selective electrode (CPBISE) (figure 3e). Here, the ion-selective sites are directly embedded in the conducting polymer. This is done by either functionalizing the conducting polymer or by immobilizing the ion-recognition sites as dopants or counter ions during the electrochemical synthesis [36].

The ISEs can be grouped using several characteristics. Bard and Faulkner [37] propose dividing membranes into solid-state membranes (sub-divided again into glass membranes and crystalline membranes as suggested by Mikhelson [38]) and to liquid and polymer (plastic) membranes. This research project will focus on plastic membranes.

The plastic membranes consist of an ionophore immobilized in a PVC matrix. In addition to the ionophore, there are lipophilic salts and plasticizers added to the membrane to enhance its physical and mechanical properties. A typical PVC-based ISM contains 33% (w/w) PVC, 66% (w/w) plasticizer, and 1.0% (w/w) ionophore, and a minute amount of lipophilic salt [39]. These components are dissolved in a volatile solvent, typically tetrahydrofuran (THF). For solid-state electrodes, a known amount of the membrane cocktail is drop-casted on a suitable substrate, let THF evaporate, and conditioned in an appropriate solution prior to use. The role of each component in the ISM is discussed briefly below.

All the components in an ISM play a crucial role. The key component in the ISM is the ionophore. The role of the ionophore is to give membrane its selectivity. Ionophores can be either charged or neutral molecules that binds the target ion selectively and reversibly. Perhaps the most well-known ionophore is valinomycin (figure 4), a neutral carrier which is highly selective for K^+ ions [40]. A good ionophore must retain a high binding affinity towards the target ions and a weak binding affinity towards interfering ions.

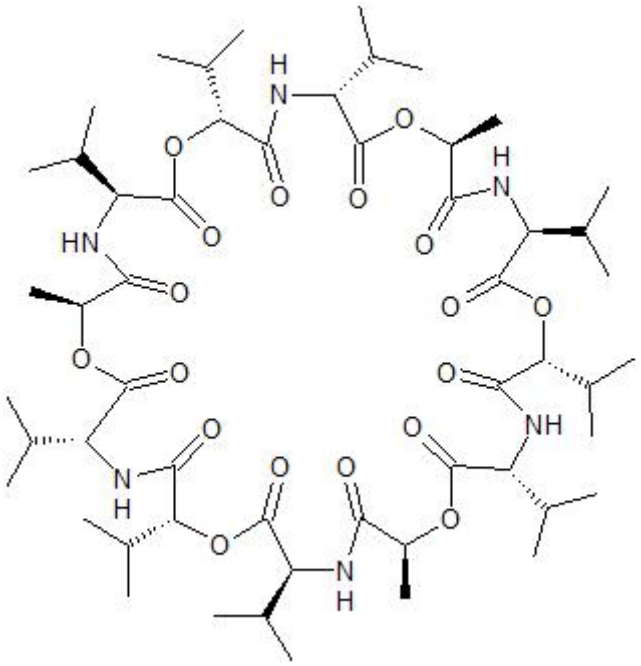


Figure 4: Molecular Structure of Valinomycin (Drawn with ChemSketch Freeware)

An ionophore immobilized in a PVC matrix alone is inadequate to construct an ISE with a theoretical response. While an ionophore with a high binding affinity towards the analyte is preferred, a too strong binding affinity can lead to undesired results such as the co-extraction of an ion with the opposite charge that of the analyte from the aqueous phase, consequently losing the permselectivity of the membrane (i.e. the Donnan exclusion failure) [41]. The ISEs experiencing the Donnan failure exhibit a less than ideal response in higher concentrations of the analyte since there are not enough binding sites left to bind the analyte due to interference from counter ions. In order to prevent the Donnan failure, a suitable lipophilic salt is added to the ISM. The lipophilic salt being introduced to the membrane dissociates within the membrane phase into two parts; a hydrophobic and a hydrophilic ion. The hydrophilic ion has the same charge as the analyte. These dissociated ions function as ion exchangers within the membrane to preserve the demand for electroneutrality thus maintaining the permselectivity and the Donnan exclusion. Especially in the cases where a neutral carrier (such as valinomycin) is used as the ionophore, addition of lipophilic salt is of utmost importance. When a charged ionophore is used, the ionophore itself can induce some ion-exchange capacity in the polymeric membrane [29]. However, it has been observed that the presence of lipophilic salts is still beneficial even in such cases and the early ISMs containing neutral ionophores but no lipophilic salts were found to function as a result of impurities present in the PVC matrix acting as ion-exchangers [41]. In a nutshell, lipophilic salts also play a role in selectivity of the membrane. The selectivity of membranes containing only lipophilic ion exchangers (i.e. in the absence of

an ionophore) follows the Homeister series which ranks cations and anions according to their lipophilicity [42]. It has also shown that the addition of lipophilic salts results in lower membrane electrical resistance and detection limits, and enhance sensitivity of carriers with poor extraction capability. It must be noted, however, that the concentration of these lipophilic salts (the molar ratio between the lipophilic salt and the ionophore) must be controlled as excessive concentrations lead to dramatic changes in the selectivity of the membrane [43].

The largest component by weight in ISMs is the plasticizer and typically the mass ratio of PVC to plasticizer is 1:2 [38]. The plasticizers play several key roles in ISMs. They act as a solvent for ionophores within the membrane and improves the mechanical stability of the membrane. Since the PVC has a relatively high glass transitioning temperature (T_g), typically between 85-102 ^oC depending on impurities, a plasticizer is needed to impart elasticity to the membrane [29].

1.2 Conducting Polymers

Polymers have almost always been thought of as insulators. However, the notion of increasing the electrical conductivity of plastics by adding conductive materials into resin matrices originated from conductive rubber mixed with carbon black in the late 19^{th} century [44]. Since then, conducting polymers have passed through several milestones. In 1930, conductively filled polymers were first made for the prevention of corona discharge [45]. In 1973, it was discovered that the inorganic polymer polysulfur nitride is a metal whose conductivity at room temperature is comparable to that of copper [46]. It also becomes a superconductor below a critical temperature of 0.3 Kelvins [47]. This discovery was particularly significant in the sense that it reinforced the idea of the existence of highly conducting polymers which inspired vast amount of research in the field. Polysulfur nitride, however, has physical properties, such as its explosive nature, which prevented it from being used for commercial purposes. It was further observed that its conductivity at room temperature can be enhanced by an order of magnitude by exposing it to oxidizing agents such as bromine. By doing so, the oxidized form of polysulfur nitride conducts electricity through a polymeric cation and the charge neutrality is maintained by the reduced form of the oxidizing agent (e.g. bromide ions) [48].

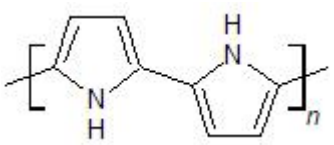
Meanwhile, a Japanese chemist, Hideki Shirakawa was pleasantly surprised by an accidental discovery while studying the polymerization of acetylene. Once, thousand times more than the usual amount of catalyst was accidentally added into a research vessel containing acetylene

which created a silvery film. A coincidental meeting between Shirakawa and Alan G. MacDiarmid, who back then was working with polysulfur nitride, led to the discovery of conducting polymers. The two of them along with Alan J. Heeger later discovered that the conductivity of polyacetylene can be increased by billion times by oxidizing it with iodine vapor. In mid-1977, Shirakawa, MacDiarmid, and Heeger published their discovery in the article "Synthesis of electrically conducting organic polymers: Halogen derivatives of polyacetylene $(CH)_n$" [49] which essentially began the modern era of conducting polymers. The importance of this discovery was showcased when they jointly received the Nobel Prize in chemistry "for the discovery and development of conductive polymers" in the year 2000 [49].

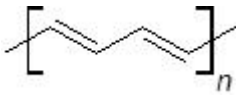
The conducting polymers are often categorized into four main groups [50].

- Conductively filled polymers which are composites of insulating polymers and conductive materials such as carbon black. Their conductive properties are due to the conductive particles in contact with each other.
- Ionically conductive polymers whose conductive properties are relied on charge carrying ions.
- Redox polymers in which the charges are propagated through the electron hopping process.
- Conjugated polymers (electrically conducting polymers) where the delocalized π electron backbone is responsible for the electrical properties of the polymer.

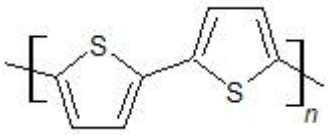
After the discovery of conducting polymers, polyacetylene attracted a great deal of scientific attention. However, several issues including its low chemical stability restricted its value for practical applications [51]. Thus, some other conducting polymers (figure 5) which include but not limited to poly(3,4-ethylenedioxythiophene) (PEDOT), polypyrrole (PPy), polythiophene (PT), polyaniline (PANI), poly(paraphenylene) (PPP), and poly(p-phenylene vinylene) (PPV) have received a great deal of attention from both scientific and commercial communities [52].



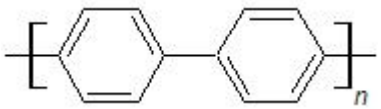
Polypyrrole



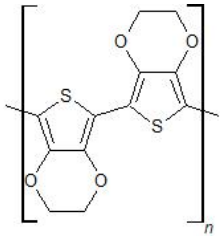
Trans-polyacetylene



Polythiophene



Poly(paraphenylene)



Poly(3,4-ethylenedioxythiophene)

Figure 5: Molecular structures of some common conjugated polymers in their neutral state. (Drawn with ChemSketch Freeware)

Apart from opening a whole new area of research, the primary advantage of conducting polymers comes from a pragmatic stand point. They are easy to synthesize, less expensive than their traditional counterparts, and they have a wide variety of practical applications. Multiple applications have been developed for conducting polymers starting from early 1980s, some of which are thin film transistors, polymer light emitting diodes, corrosion resistance, electromagnetic shielding, chemical sensors, supercapacitors, and electrochromic devices [53].

1.3 Doping in Conducting Polymers

In terms of electron flow through solid matter, all materials can be categorized in to three groups: metals, semiconductors, and insulators. To understand the difference between the three groups, the band theory of solids is a useful tool. While every solid contains electrons, the way they behave in an applied electric field is drastically different. The electrons are arranged in energy bands, separated by a region where no wave-like electron orbitals exist [54]. This region void of any electronic energy levels is termed the band gap (E_g) [55]. The bands are a result of overlapping many molecular orbitals where energy can be considered as continuous. At zero

Kelvin, the highest range of energies where electrons are present is called the valence band and the lowest range of vacant electronic states is called the conduction band.

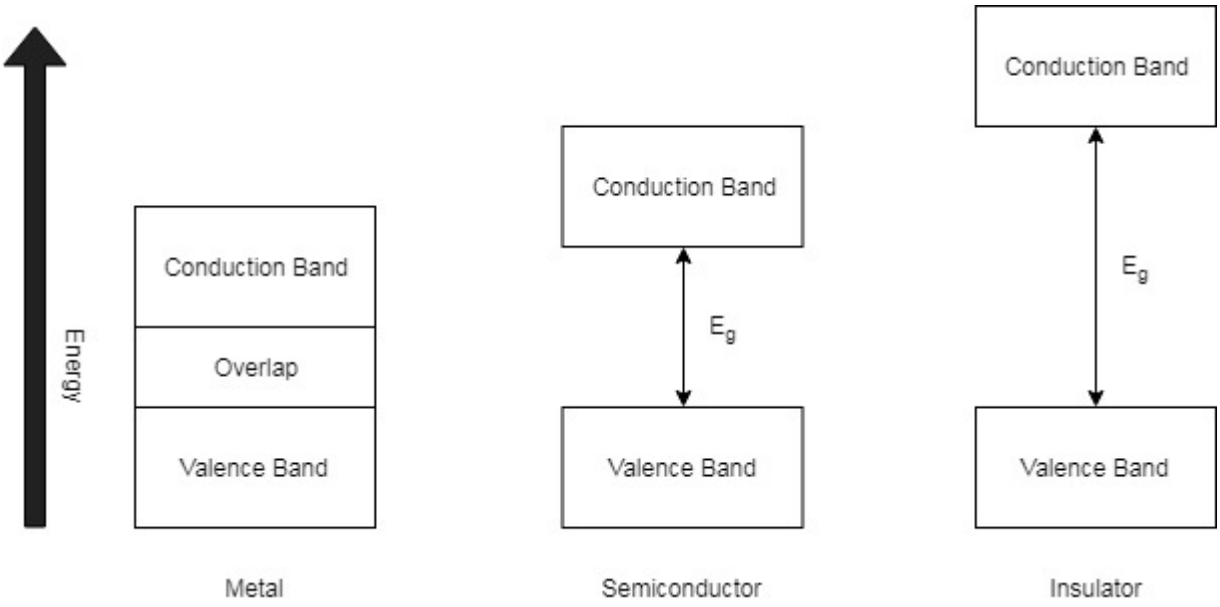


Figure 6: Energy band gap in metals, semiconductors, and insulators

As shown in figure 6, the conduction band and the valence band are overlapped in metals unlike in the case with semiconductors and insulators. In semiconductors and insulator, electrons require extra energy to move from the valence band to the conduction band. In addition, fully occupied nor completely empty bands cannot conduct electricity [56]. Since the bands overlap in metals, and they have partially filled bands, they can easily conduct electricity. The difference between the semiconductors and the insulators is the magnitude of band gap. Semiconductors have a much narrower band gap (figure 6) compared to that of insulators. Thus, the electrons in the valence band in semiconductors can be excited into the conduction band, creating partially filled bands, using an external energy source (for example, thermal excitation).

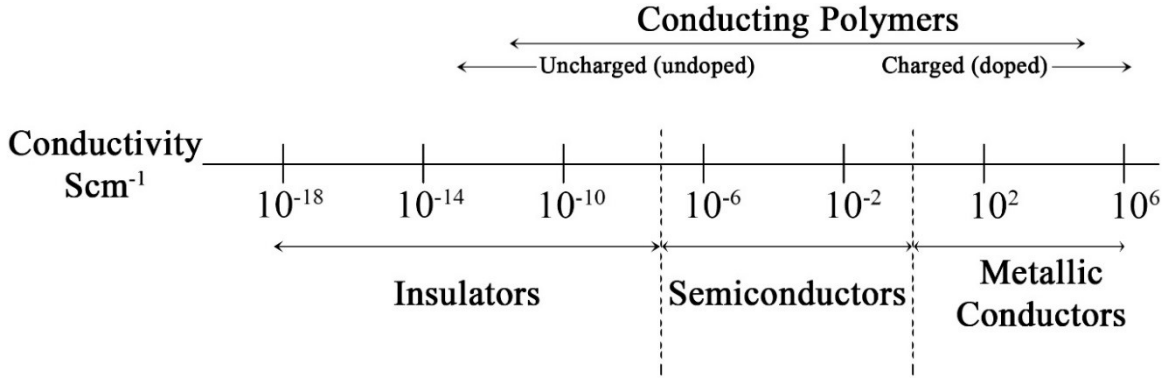


Figure 7: Conductivity of metals, semiconductors, and insulators. Adapted from [57]

In their neutral state, conducting polymers are insulators or semiconductors as illustrated in figure 7. When it comes to conjugated polymers, the conductivity is increased by a process called “doping”. The conducting polymers can be doped in two separate ways. They can be either p-doped (oxidized, i.e. electrons are extracted from the highest occupied molecular orbital (HOMO) of the valence band) or they can be n-doped (reduced, i.e. addition of electrons to the lowest unoccupied molecular orbital (LUMO) of the conduction band) [52].

The doping of conjugated polymers can be achieved either chemically or electrochemically. In chemical doping, the polymer (P) is either oxidized (Eqn. 3) by an electron acceptor (A) or reduced (Eqn. 4) by an electron donor (D). The charge neutrality is maintained by the reduced form of the electron acceptor in case of p-doping or the oxidized form of the electron donor in case of n-doping.

Chemical p-doping	$P + A \rightarrow P^+A^-$	(Eqn. 3)
Chemical n-doping	$P + D \rightarrow P^-D^+$	(Eqn. 4)

In electrochemical doping, an electrical potential is applied to the conjugated polymer, oxidizing (Eqn. 5) or reducing (Eqn. 6) it, depending on the applied potential. Here, the charge neutrality is maintained by simultaneous addition of doping ions (X^- or N^+) and the redox state of these doping ions remain unchanged during the process.

Electrochemical p-doping	$P + X^- \rightarrow P^+X^- + e^-$	(Eqn. 5)
Electrochemical n-doping	$P + N^+ \rightarrow P^-N^+ - e^-$	(Eqn. 6)

Therefore, this doping process induces mobile positive and negative charges within the polymer chain. In other words, looking back at the band theory, this process of addition or extraction of electrons creates partially filled new energy bands within the band gap, thus increasing the conductivity of the material by several orders of magnitudes. At high levels of doping, the conductivity of conjugated polymers can be comparable to that of metals.

In this work, a conjugated polymer, PEDOT electrochemically doped with chloride, has been utilized as ion-to-electron transducer in carbonate and bicarbonate ion-selective electrodes. PEDOT is a remarkably promising conducting polymer with low oxidation potential, high electrical conductivity, excellent environmental stability, and a low band gap [58].

Chapter 2: Methods

2.1 Potentiometry

Potentiometry, as the name suggests, measures the potential of an electrode system. This technique is widely employed in all kinds of electrochemical analysis. The system consists of two electrodes (a reference electrode and an indicator electrode), a potentiometer, and a solution of interest. The potential of the indicator electrode (or the ISE) depends ideally only on the activity of the ion the electrode is selective for. The reference electrode, on the other hand, should provide a very steady potential (ideally a constant potential) throughout the measurement process irrespective of the analyte ion (or any other ion) activity and independent of temperature. Therefore, any change in the cell potential can be attributed to the analyte's effect on the ion-selective electrode's potential. Then using a potentiometer, the potential (or the Electromotive Force, EMF) between the reference and the working electrodes is measured under zero current conditions. A typical potentiometric setup using conventional ISEs is shown in the figure 8 below.

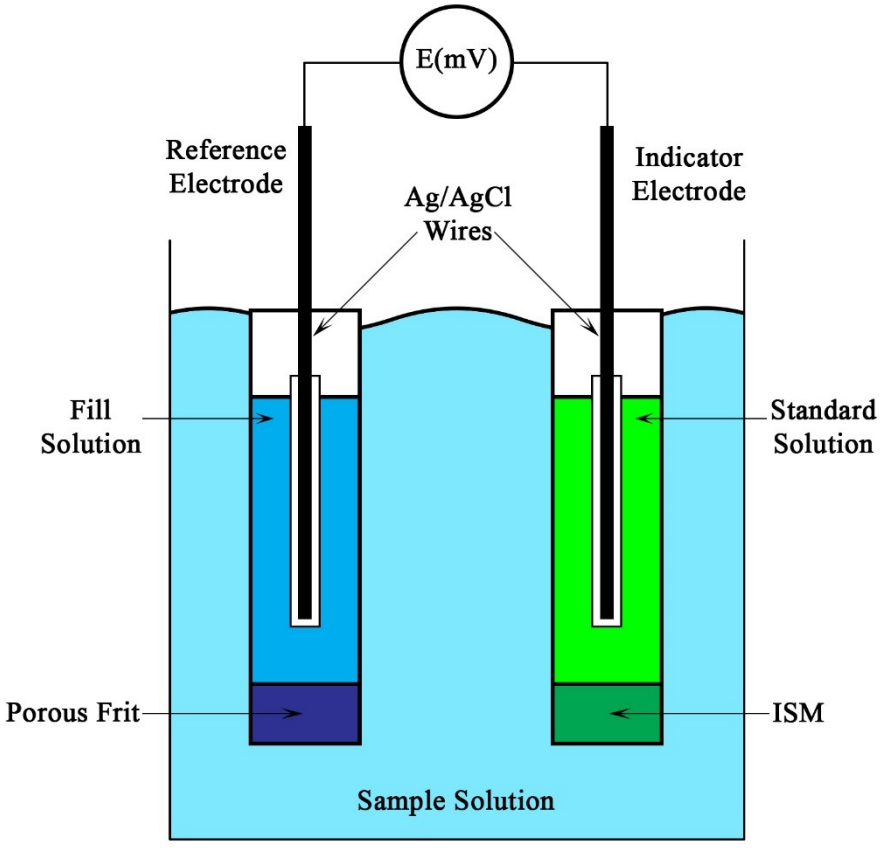


Figure 8: Schematic Diagram of a Typical Potentiometric Setup Using Conventional Electrodes

The magnitude of the potential in an electrochemical cell is given by,

$$E_{cell} = E_{ind} - E_{ref} + E_j \qquad \text{(Eqn. 7)}$$

Where E_{ind} is the potential of the indicator electrode, E_{ref} is the potential of the reference electrode, and E_j is the liquid-junction potential (figure 9).

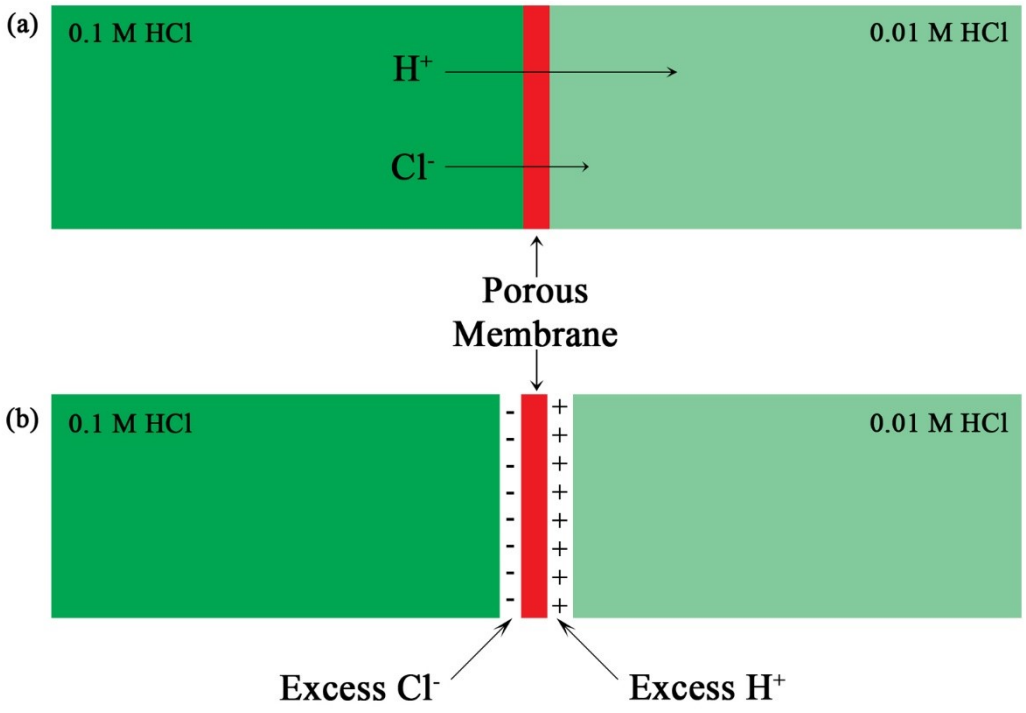


Figure 9: Formation of liquid junction potential. Reproduced from Analytical Chemistry 2.1 [59]

A junction potential is formed at the boundary between two ionic solutions provided that there is a difference in the concentrations and ionic mobilities. As shown in figure 9a, H^+ has a higher ionic mobility (represented by the longer arrow) compared to that of Cl^-. Thus, it creates an excess of H^+ on one side of the membrane and an excess of Cl^- on the other side of the membrane (figure 9b). The magnitude of this junction potential can be as high as 30-40 mV. For example, a junction potential as high as 33.09 mV has been reported at the interface between 0.1 M HCl and 0.1 M NaCl solutions [60]. While it's not realistic attempting to completely eliminate the junction potential, it can be minimized by using a high concentration of salt in the salt bridge and using inner filling solutions which contains cations and anions with similar mobilities. For example, KCl, Lithium Acetate (LiOAc), NH_4NO_3 are often used as inner filling solutions [37, 38]. However, it is important to note that there is always a small

junction potential (residual junction potential, generally of unknown magnitude) present. The liquid junction potential can be estimated by the Henderson equation [37] (Eqn. 8).

$$E_j = \frac{\sum_i^n \frac{|z_i|u_i}{z_i}[C_i(\beta)-C_i(\alpha)]}{\sum_i^n |z_i|u_i[C_i(\beta)-C_i(\alpha)]} \frac{RT}{F} ln \frac{\sum_i^n |z_i|u_i C_i(\alpha)}{\sum_i^n |z_i|u_i C_i(\beta)} \quad \text{(Eqn. 8)}$$

Where z_i is the charge of the ion i, u_i is the ionic mobility and C_i is the concentration. α and β represent the two different liquid phases. As shown in equation 7, the junction potential also contributes to the overall cell potential. Since in many cases the junction potential is unknown, directly calculating the analyte concentration is not accurate. Therefore, in quantitative analysis, standardization methods are utilized [59].

The relationship between the electric potential and the logarithm of the activity of the analyte in the sample solution is illustrated by the Nernst equation [61] (Eqn. 9).

$$E = E^0 + \frac{RT}{z_i F} ln\, a_i(aq) \quad \text{(Eqn. 9)}$$

Where E is the measured cell potential, E^o is the standard potential (dependent upon the characteristics of both indicator and reference electrodes), R is the universal gas constant, T is the absolute temperature, F is the Faraday constant, z_i is the charge of analyte (or the ion of interest) and a_i is the activity of the ion of interest in the solution. Since R, and F are constants, for an ion with one charge, the Nerstian slope has a value of 59.2/z mV at 25 °C. This value is negative for anions and positive for cations.

The measured cell potential depends on the activity of the analyte (effective concentration) instead of the concentration. The activity of an ion (i) and its concentration are related by the following equation (Eqn.10).

$$a_i = \gamma_i c_i \quad \text{(Eqn. 10)}$$

Where γ_i is the activity coefficient. The activity coefficient is dependent upon the ions present in the solution and the total ionic strength (μ). It can be calculated by the extended Debye-Huckel equation (Eqn. 12) [62]. The ionic strength is calculated using Eqn. 11

$$\mu = \frac{1}{2}\sum_i c_i z_i^2 \quad \text{(Eqn. 11)}$$

$$log\gamma_i = \frac{-0.50925 \times z_i^2 \times \sqrt{\mu}}{1+3.2864 \times \propto_i \times \sqrt{\mu}} \quad \text{(Eqn. 12)}$$

Where α_i is the size of the ion of interest in nanometers.

2.2 Separate Solution Method

While ideal indicator electrodes (ISE) only respond to the ion they are selective for, as mentioned before, realistically this is often not the case. Almost all real-life samples contain diverse types of ions. Therefore, in order to obtain reliable results, it is important to take into account the possibility of these ions interfering with the measurement. The potentiometric selectivity coefficients can be determined by using various methods, some of which are Separate Solution Method (SSM), Mixed Solution Method (MSM), Fixed Primary Ion Method (FPM), and Fixed Interfering Ion Method (FIM). In this project, SSM is employed to determine the selectivity of carbonate and bicarbonate electrodes against four anions; chloride, nitrate, sulphate, and salicylate.

In SSM, the potential of an ISE is measured in the primary ion (A) solution followed by the interfering ion (B) solution separately. Then the selectivity coefficient is calculated by the extended Nikolskii-Eisenman equation (Eqn. 13) [63].

$$logK_{A,B} = \frac{(E_B - E_A)z_A F}{RT\ ln10} + \left(1 - \frac{z_A}{z_B}\right) loga_A \qquad \text{(Eqn. 13)}$$

Where E_A and E_B are the measured potentials (in respective solutions) and z_A and z_B are charges of the primary ion and the interfering ion respectively.

If log $K_{A,B} < 1$, the ISE responds more selectively towards the primary ion, if log $K_{A,B} > 1$, the ISE responds more selectively towards the interfering ion, and if log $K_{A,B}$ has a value equal to or close to 1 the ISE is almost equally sensitive to the interfering ion as to the primary ion.

The SSM, along with other methods, were first recommended by IUPAC in 1976 [64]. These recommendations have been quite popular and more than 60% of the papers published between 1976 and 1988 followed IUPAC recommendations [65]. However, the IUPAC methods based on the Nikolskii-Eisenman equation have been under constant criticism due to the fact that these methods result in overestimated, measuring condition dependent, and biased selectivity coefficients [66]. This problem stems from the fact that the Nikolskii-Eisenman equation assumes a Nernstian slope for both the primary ion and the interfering ion, which is usually not true for all real-life cases [63].

Nonetheless, this project will utilize the SSM, due to its simplicity, while acknowledging all of its limitations. Since the bicarbonate electrodes are at their initial stage of development, a rough

estimation of selectivity should suffice for the time being. Once the electrodes have been optimized, alternative methods of determining selectivity can be considered.

Chapter 3: Experimental Design

3.1 Materials

Table 1: Reagents used in this Thesis

Reagent	Molar Mass ($gmol^{-1}$)	Purity	Make	Lot Number
Carbonate & Bicarbonate IMS Cocktail				
O-NPOE[I]	251.32	≥ 99%	Sigma Aldrich	#BCBS0242V
TDMACl[II]	572.47	Unknown	Sigma Aldrich	#BCBR4272V
DOA[III]	370.57	≥ 99%	Sigma Aldrich	#BCBV4867
Carbonate Ionophore[IV]	1016.24	Unknown	Sigma Aldrich	#BCBK0880V
Bicarbonate Ionophore	982.53	Novel Ionophore from University of Tartu		
PVC[V]	High Molecular Weight	Unknown	Sigma Aldrich	#E371469/1V
THF[VI]	72.11	99.5%	Sigma Aldrich	#BCBN5103V
EtOAc[VII]	88.11	>99.5%	Fluka	#72130
Other Solutions				
NaCl	58.44	≥ 99.5%	Merck	#K43925404 241
$NaHCO_3$	84.001	99.5-100.5%	Sigma Aldrich	#SLBT8842
Na_2CO_3	105.99	≥ 99.0%	Sigma Aldrich	#BCBT4969
$NaH_2PO_4.2H_2O$	156.01	98-100.5%	Merck	#632 K2711145
Na_2HPO_4	141.96	99.9%	J.T. Baker	#9327710006
KCl	74.55	99.5%	Sigma Aldrich	#SZBE0804V
EDOT[VIII]	142.18	Unknown	Sigma Aldrich	#MKAA1643
TRIZMA[IX]	121.1	99.9%	Sigma Aldrich	#109H5401
Na_2SO_4	142.04	99%	Riedel-de Haen	#31481
$NaNO_3$	84.99	99.5%	Sigma Aldrich	#SZBA1670
Sodium Salicylate	160.10	≥ 99.5%	Sigma Aldrich	#WXBC4921V

- I - 2-Nitrophenyl octyl ether
- II - Tridodecylmethylammonium chloride
- III – Bis(2-ethylhexyl) adipate
- IV - N,N-Dioctyl-3α,12α-bis(4-trifluoroacetylbenzoyloxy)-5β-cholan-24-amide (Carbonate Ionophore VII)
- V – Poly(vinyl chloride) – High Molecular Weight
- VI – Tetrahydrofuran
- VII – Ethyl Acetate
- VIII – 3,4-ethylenedioxythiophene
- IX – Tris(hydroxymethyl)aminomethane

Instruments

- Lawson Labs EMF 16 Channel Multi-voltmeter (Malvern, PA, USA)
- Autolab PGSTAT 20 (Eco Chemie, The Netherlands)
- Mettler Toledo AG204 Analytical Balance
- Metrohm 846 Dosing Interface & Metrohm 800 Dosino
- Metrohm Single Junction Ag/AgCl/3M KCl Reference Electrode
- Thermo Scientific Orion 8101BNWP pH electrode

3.2 Experimental Procedure

Construction of Electrodes

The following electrodes were prepared for this study and the ion-selective membrane composition is given below in table 2.

- Two conventional bicarbonate selective electrodes
- Two conventional carbonate selective electrodes
- Two solid-contact bicarbonate selective electrodes
- Two solid-contact carbonate selective electrodes

Table 2: Ion-Selective Membrane Compositions

Reagent	Composition (w/w)	Calculated Mass (mg)	Measured Mass (mg)
Bicarbonate Electrodes			
Bicarbonate Ionophore	2%	12.7	12.7
O-NPOE	65%	414.3	414.8
TDMACl	50% relative to ionophore	3.7	3.6
PVC	33%	210.3	210.7
Carbonate Electrodes			
Carbonate Ionophore	5.1%	28.01	28.3
TDMACl	1.2%	6.59	6.6
DOA	56.8%	312.00	313.2
PVC	36.9%	202.69	202.8

For bicarbonate electrodes, all the components were dissolved in 3.5 mL of THF such that the THF concentration is 83% (w/w) and ISM components ~17% (w/w). The components were put into a small glass vial with a Teflon cap, mixed thoroughly using a vortex mixture, and further allowed to mix overnight on an orbital shaker platform.

Then 2.5 mL of the cocktail was poured into a small Teflon ring placed on a Teflon platform and let THF evaporate. From the dried membrane, two disks (5 mm diameter) were punched and used as the ion-selective membrane in Philips IS561 (conventional electrode bodies that contain Ag/AgCl wire, Möller glasbläserei, Zürich, Switzerland). For inner filling solution, 0.01 M NaCl and 0.01 M $NaHCO_3$ were used.

To prepare the solid-contact electrodes, two glassy carbon electrodes were polished using sand paper (from 200 to 4000 increasing grit size), then diamond paste (15 µm, 9 µm, 3 µm, and 1 µm), and finally 0.3 µm Al_2O_3 paste. The electrodes were then tested with cyclic voltammetry for any contaminations. The solid-contact for each electrode was applied by electropolymerization (galvanostatic mode / 0.0141 mA / 710 seconds) in 0.01 M EDOT and 0.1 M KCl. The electrodes with the conducting polymer were conditioned in 0.01 $NaHCO_3$ for 24 hours and dried for another 24 hours. Finally, 100 µL of the membrane cocktail was drop-casted on each electrode.

Preparation of carbonate electrodes were carried out in an analogous manner. However, the ISM components were dissolved in 1.875 mL of THF and 1.125 mL of EtOAc (5:3 volume ratio). Inner filling solution for conventional carbonate electrodes consists of 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_3, and 0.01 M NaCl. The electrodes were conditioned in 0.01 M Na_2CO_3.

When not in use, all electrodes were kept in their respective conditioning solutions.

Electrode Calibration

The following calibrations were performed.

- Conventional Electrodes in Carbonate
- Conventional Electrodes in Bicarbonate
- Solid-State Electrodes in Carbonate
- Solid-State Electrodes in Bicarbonate
- Conventional Electrodes in Carbonate (Tris Buffer pH ~8.6)
- Conventional Electrodes in Bicarbonate (Tris Buffer pH ~8.6)
- Solid-State Electrodes in Carbonate (Tris Buffer pH ~8.6)
- Solid-State Electrodes in Bicarbonate (Tris Buffer pH ~8.6)
- Conventional Electrodes in Carbonate (Boiled Water)
- Conventional Electrodes in Bicarbonate (Boiled Water)
- Solid-State Electrodes in Carbonate (Boiled Water)
- Solid-State Electrodes in Bicarbonate (Boiled Water)

All calibrations were performed with a Lawson Labs EMF 16 channel multi-voltmeter and a Metrohm Single Junction Ag/AgCl/3M KCl Reference Electrode. A Thermo Scientific Orion 8101BNWP pH electrode (calibrated previously) was also included in the setup. Solid-state electrodes and conventional electrodes were calibrated separately. Half decade dilutions were carried out automatically using Metrohm 846 Dosing Interface and a Metrohm 800 Dosino. The calibration range was six and a half decades starting from 0.1 M carbonate or bicarbonate.

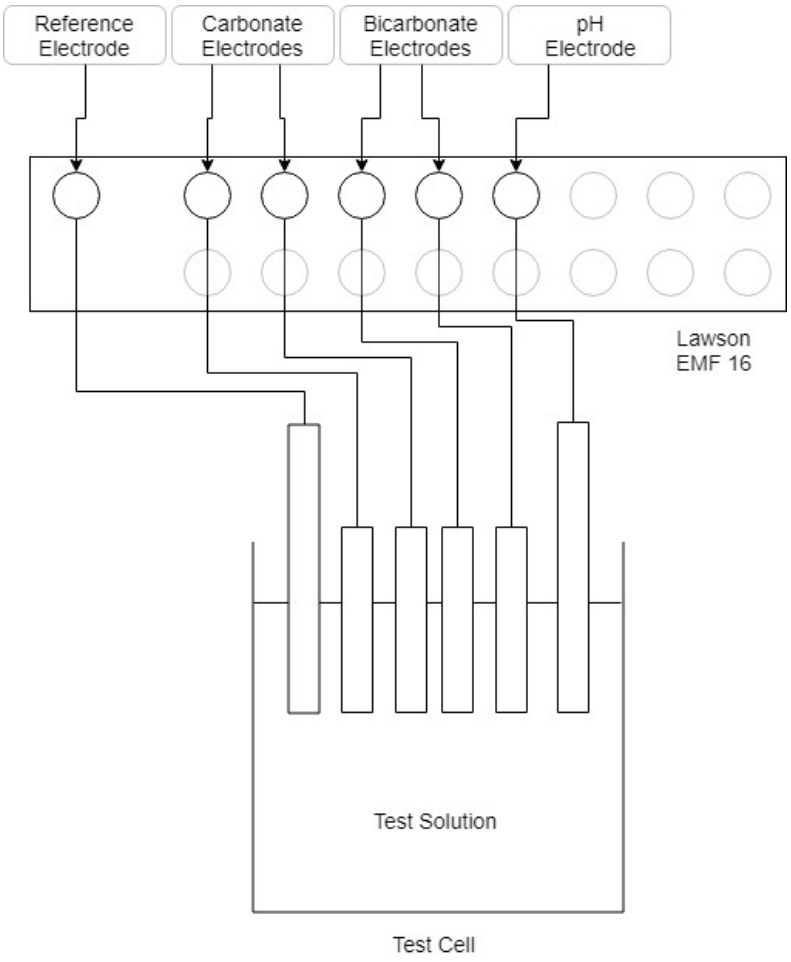


Figure 10: Typical Test Setup (Automatic Dilution Unit Not Shown)

For calibrations in Tris buffer, a 0.1 M buffer was prepared, and the pH was adjusted to 8.6 pH units by adding drops of concentrated H_2SO_4. Then the carbonate and bicarbonate solutions were prepared using the buffer solution.

To imitate a closed system for carbonic acid species, a set of calibrations were also performed with calibration solutions prepared using boiled water. Deionized water was boiled for approximately 30 minutes to remove dissolved CO_2, and let it cool down to room temperature in a sealed container. Calibration solutions were prepared using the boiled water with minimum possible contact with the atmospheric air. The measuring cell with the calibration solution was

purged with argon gas for approximately 5 minutes and during measurements argon gas was allowed to blow on top of the liquid layer to prevent atmospheric CO_2 from dissolving.

Selectivity Measurements

The selectivities were measured using the separate solution method with 0.01 M sodium salts of the analytes (nitrate, sulphate, chloride, and salicylate). Selectivities against carbonate and bicarbonate in both solid-state electrodes and conventional electrodes were measured.

Sample Measurement

Electrodes were calibrated again in bicarbonate solution (Log [DIC] = -2 to Log [DIC] = -4.5) (DIC = Dissolved Inorganic Carbon) and the bicarbonate level in blood serum sample was measured. All the calibration solutions were prepared by using boiled deionized water following the same procedure as earlier.

Following sample was measured.

- Thermo Scientific Abtrol (Ref: 981044) – bicarbonate (36 mmol/L)

Chapter 4: Results and Discussion

4.1 Carbonic Acid Species Equilibria

As shown in figure 1, the molar fractions of carbonic acid species are dependent upon the pH. This pH dependency makes determination of carbonic acid species relatively more complicated than other ions that dissociates completely in aqueous media. The availability of free ions is determined by several dynamic equilibria (figure 11).

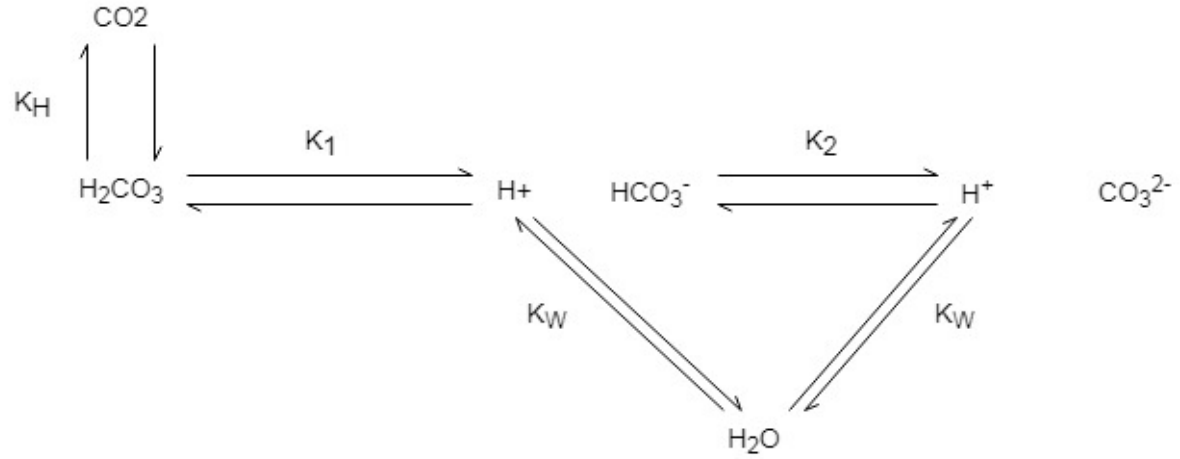


Figure 11: Carbonic Acid Species Equilibria

K_1 and K_2 are the first and the second dissociation constants of carbonic acid, K_W is the self-ionization constant of water, and K_H is the Henry's Law constant for CO_2.

$$K_H = \frac{[H_2CO_3]}{p_{CO_2}} \qquad \text{(Eqn. 14)}$$

$$K_1 = \frac{[H^+]\times[HCO_3^-]}{[H_2CO_3]} \qquad \text{(Eqn. 15)}$$

$$K_2 = \frac{[H^+]\times[CO_3^{2-}]}{[HCO_3^-]} \qquad \text{(Eqn. 16)}$$

$$K_W = [H^+] \times [OH^-] \qquad \text{(Eqn. 17)}$$

In a closed system, the atmospheric CO_2 disappears from the equation. Thus, the total amount of carbonic acid species (C_T = DIC = Dissolved Inorganic Carbon) is a conserved quantity. However, the amount of each carbonic species is dependent upon the pH. Therefore,

$$DIC = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \qquad \text{(Eqn. 18)}$$

The charge balance equation can be written as,

$$0 = [Cations] + [H^+] - [HCO_3^-] - 2[CO_3^{2-}] - [OH^-] \quad \text{(Eqn. 19)}$$

By writing each species in terms of $[H^+]$ and dissociation constants (K_1, K_2, & K_W), molar fraction of each species at any given pH can be calculated. From Eqn. 15 & 16,

$$K_1K_2 = \frac{[H^+]^2 \times [CO_3^{2-}]}{[H_2CO_3]} \quad \text{(Eqn. 20)}$$

Solving Eqn. 15 for HCO_3^-, Eqn. 20 for CO_3^{2-}, and substituting into the mass balance equation (Eqn. 18),

$$[HCO_3^-] = \frac{K_1[H_2CO_3]}{[H^+]} \quad \text{(Eqn. 21)}$$

$$[CO_3^{2-}] = \frac{K_1K_2[H_2CO_3]}{[H^+]^2} \quad \text{(Eqn. 22)}$$

$$DIC = [H_2CO_3] + \frac{K_1[H_2CO_3]}{[H^+]} + \frac{K_1K_2[H_2CO_3]}{[H^+]^2} \quad \text{(Eqn. 23)}$$

Which can be rearranged to,

$$DIC = [H_2CO_3] \times \frac{[H^+]^2 + K_1[H^+] + K_1K_2}{[H^+]^2} \quad \text{(Eqn. 24)}$$

$$[H_2CO_3] = \frac{[H^+]^2 \times DIC}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 25)}$$

This can be substituted back into Eqn. 21 & Eqn. 22.

$$[HCO_3^-] = \frac{K_1 \times [H^+] \times DIC}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 26)}$$

$$[CO_3^{2-}] = \frac{K_1K_2 \times DIC}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 27)}$$

Furthermore, the molar fraction of each species is given by,

$$\alpha_{H_2CO_3} = \frac{H_2CO_3}{DIC} = \frac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 28)}$$

$$\alpha_{HCO_3^-} = \frac{HCO_3^-}{DIC} = \frac{[H^+]K_1}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 29)}$$

$$\alpha_{CO_3^{2-}} = \frac{CO_3^{2-}}{DIC} = \frac{K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad \text{(Eqn. 30)}$$

It must be noted that the values of the carbonic dissociation constants have been under continual debate [67, 68]. Even values as low as $pKa_1 = 3.45$ has been reported [69]. Nonetheless, in this work, the following dissociation constants were used to calculate all data [70].

$pKa_1 = 6.4$

$pKa_2 = 10.3$

Since the relationship between pK_a, pK_b, and pK_W is known, the pK_b can also be calculated.

$$pK_W = pK_a + pK_b = 14 \qquad \text{(Eqn. 31)}$$

However, since the pK_b describes the equilibrium in reverse direction (relative to pKa), pK_{a1} will relate to pK_{b2} and pK_{a2} will relate to pK_{b1}. Therefore,

$pK_{b1} = 14 - pK_{a2} = 14 - 10.3 = 3.7$

$pK_{b2} = 14 - pK_{a1} = 14 - 6.4 = 7.6$

Thus, using the dissociation constants, the predicted pH of a carbonate system can be calculated when DIC is a known value.

Sodium salts of carbonate and bicarbonate were used in this thesis. Thus, the amount of sodium should also be taken into account when predicting the pH. The charge balance equation (Eqn. 19) changes into,

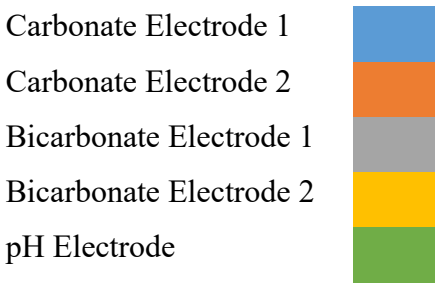
$$0 = [Na^+] + [H^+] - [HCO_3^-] - 2[CO_3^{2-}] - [OH^-] \qquad \text{(Eqn. 32)}$$

The rest of the equations remain the same. There are five equations and five unknown variables. From equations 15, 16, 17, 18, and 32, a fourth order polynomial (Eqn. 33) can be derived to predict the pH when the total amount of dissolved carbonic acid species (DIC) is known. The derivation of this equation can be found in Appendix C. For this thesis, MathCad14 software was used to predict the pH values.

$$[H^+]^4 + ([Na^+] + K_1)[H^+]^3 + (K_1K_2 + K_1[Na^+] - C_TK_1 - K_W)[H^+]^2 + (K_1K_2[Na^+] - 2C_TK_1K_2 - K_WK_1)[H^+] - K_WK_1K_2 = 0 \qquad \text{(Eqn.33)}$$

4.2 Calibration of Electrodes

In all figures that appear in the subsequent discussion, following color codes are used unless otherwise noted.



As one would expect, the conventional electrodes and solid-state electrodes follow the same trend. Therefore, only the solid-state electrodes are chosen for this discussion for clarity. Summarized data for conventional electrodes will be presented in tabulated form. All data can be found in Appendix A and all the figures that are not presented here can be found in Appendix B.

The pH electrode was calibrated with a slope of -56.3 mV/decade and an intercept of 641.0 mV. All pH measurements were calculated using this data unless otherwise noted.

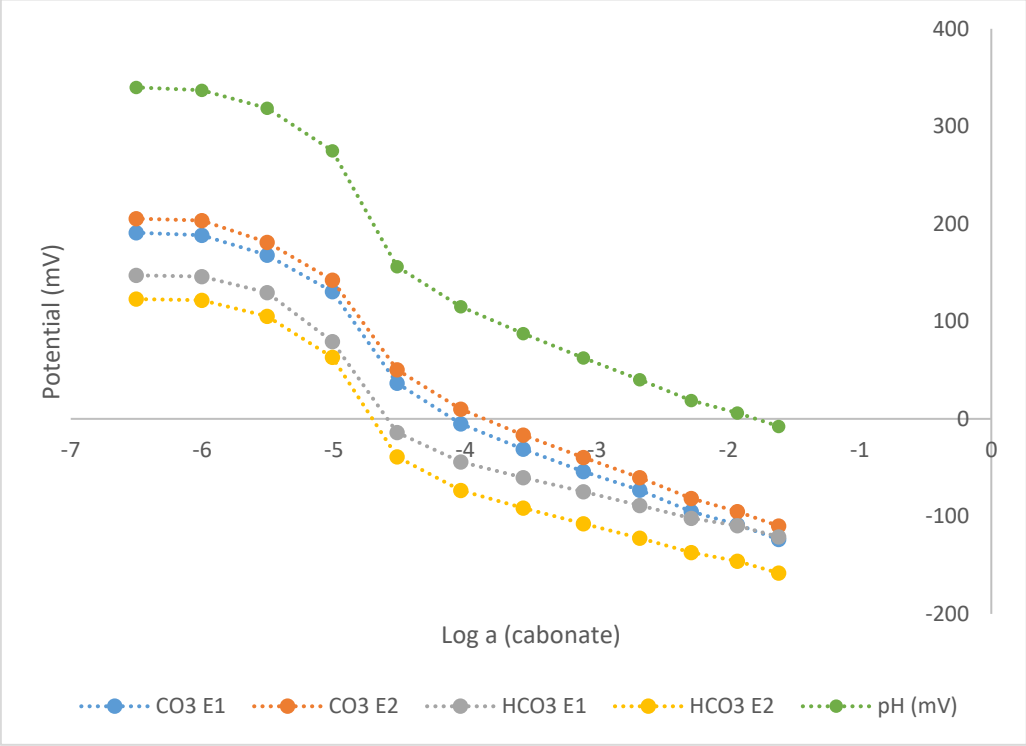


Figure 12: Solid-State Electrodes in Carbonate Solution (pH range between 11.5 - 5.3)

It can be seen in figure 12 that there is a large (~100 mV) potential jump between log [DIC] -4.5 and -5. This phenomenon is observed when the pH approaches the pKa value, in other words, near the equivalence point. The measured pH at -4.5 is 8.61 and the pH at -5 is 6.50. Thus, the difference is pH is slightly more than 2 units. From figure 1, it is evident that below pH ~8, there is very little carbonate ions left and bicarbonate becomes the dominant form. Furthermore, the slopes of the calibration are -72.3, -72.4, -61.8, and -65.2 mV/decade for carbonate and bicarbonate electrodes respectively. It is paradoxical that the carbonate electrodes (doubly charged) exhibit a higher slope than that of bicarbonate electrodes (singly charged). Thus, it is apparent that the equilibria need to be considered.

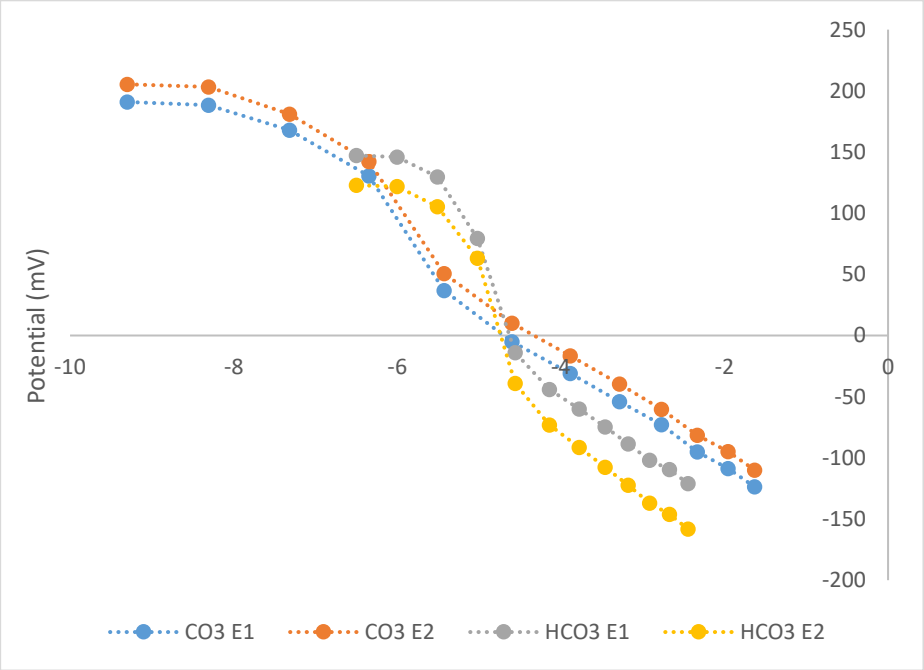


Figure 13: Solid-State Electrodes in Carbonate Solution (With Calculated Equilibria) (pH range 11.5 – 5.3)

The experimental data points are connected with dotted lines to improve the data readability and the visibility of apparent non-linearity in some regions.

In figure 13, the carbonic acid equilibria are taken into account. The slopes are -46.0, -46.1, -76.1, and -80.1 mV/decade for carbonate and bicarbonate electrodes, respectively. The carbonate electrodes are plotted against the calculated activity of carbonate and the bicarbonate electrodes are plotted against the calculated activity of bicarbonate. All figures from this point onwards are plotted similarly (hence no x-axis label). The paradoxical situation that was observed earlier has been now resolved. The carbonate electrodes exhibit a smaller slope compared to that of bicarbonate electrodes, although the slopes are still far from being ideal. Furthermore, the effective concentration of carbonate is much lower as expected at this pH.

Since the pH was also measured, the equations 29 & 30 can be used to calculate the actual concentration (therefore activity) of both carbonate and bicarbonate ions.

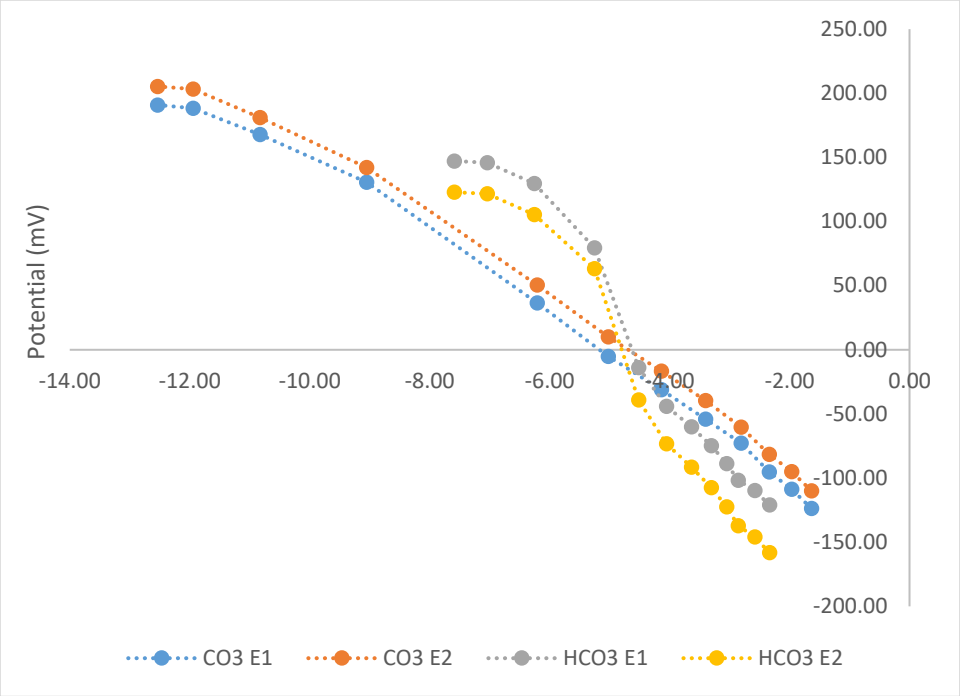


Figure 14: Solid-State Electrodes in Carbonate Solution (With Equilibria and pH) (pH range 11.5 – 5.3)

Once the measured pH is also considered (figure 14), the results become more desirable. The slopes are -29.6, -29.6, -57.9, -60.8 mV/decade. All the slopes now exhibit close to Nernstian behavior. Moreover, the carbonate electrodes are linear even at 10 orders of magnitude. However, the linear range of bicarbonate electrodes is much narrower.

Table 3: Equlibria and pH data for Solid-State Electrodes in Carbonate Solution. All concentrations are in mol/L

DIC	Log [C]	Calculated from Equilibria			pH Measured	Δ pH	Calculated from Equilibria and Measured pH		Log a_{HCO3-}	Log a_{CO32-}
		$[HCO_3^-]$	$[CO_3^{2-}]$	pH			$[HCO_3^-]$	$[CO_3^{2-}]$		
1.00E-01	-1	4.52E-03	9.55E-02	11.65	11.52	0.13	6.02E-03	9.40E-02	-2.34	-1.64
3.16E-02	-1.5	2.50E-03	2.91E-02	11.40	11.28	0.12	3.19E-03	2.84E-02	-2.58	-1.96
1.00E-02	-2	1.36E-03	8.64E-03	11.13	11.05	0.08	1.59E-03	8.41E-03	-2.86	-2.34
3.16E-03	-2.5	7.22E-04	2.44E-03	10.86	10.67	0.19	9.87E-04	2.17E-03	-3.05	-2.81
1.00E-03	-3	3.68E-04	6.32E-04	10.57	10.28	0.29	5.32E-04	4.68E-04	-3.30	-3.40
3.16E-04	-3.5	1.74E-04	1.42E-04	10.24	9.83	0.41	2.40E-04	7.66E-05	-3.64	-4.14
1.00E-04	-4	7.42E-05	2.58E-05	9.87	9.34	0.53	9.06E-05	9.32E-06	-4.05	-5.03
3.16E-05	-4.5	2.80E-05	3.66E-06	9.45	8.62	0.83	3.09E-05	5.97E-07	-4.51	-6.21
1.00E-05	-5	9.57E-06	4.29E-07	8.98	6.50	2.48	5.83E-06	8.72E-10	-5.25	-9.05
3.16E-06	-5.5	3.12E-06	4.54E-08	8.49	5.73	2.76	5.99E-07	1.50E-11	-6.26	-10.83
1.00E-06	-6	9.95E-07	4.63E-09	8.00	5.40	2.60	9.86E-08	1.16E-12	-7.04	-11.95
3.16E-07	-6.5	3.16E-07	4.66E-10	7.50	5.35	2.15	2.82E-08	2.95E-13	-7.59	-12.54

The difference between the calculated (predicted) and the measured pH becomes significant at lower concentration (table 3). Analogous to what was observed earlier, around pH ~5 molar fraction of bicarbonate is much lower, and the carbonic acid becomes the dominant form.

All the calibration figures from this point onwards will have equilibria and measured pH taken into account. Each species will be plotted against its calculated effective concentration (activity).

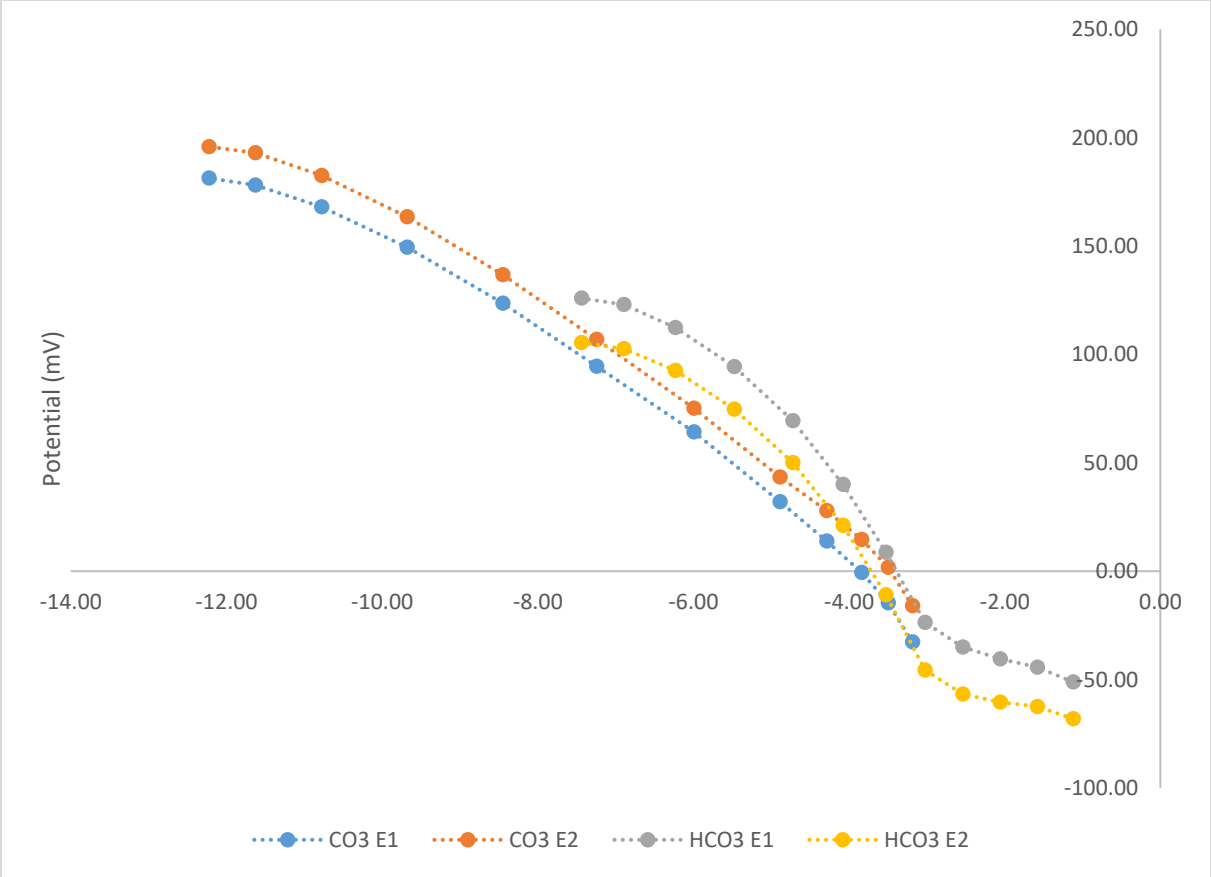


Figure 15: Solid-State Electrodes in Bicarbonate Solution (pH range 8.5 – 5.5)

In bicarbonate solutions, however, the performances of the electrodes are not as ideal (figure 15). The slopes are -23.7, -23.6, -32.9, and -32.7 mV/decade. The linearity of the carbonate electrodes is still preserved. It is also evident that the response of the bicarbonate electrodes at higher concentrations is restricted. It can be hypothesized that these electrodes are experiencing the Donnan exclusion failure since this phenomenon was also observed in conventional bicarbonate electrodes. This can be clearly seen in the time response graph (figure 16). The electrodes start responding around 10^{-3} M HCO_3^-. If the limited range from 10^{-3} M to 10^{-5} M is considered, the slopes are improved to -48.1 and -48.8 mV/decade, respectively.

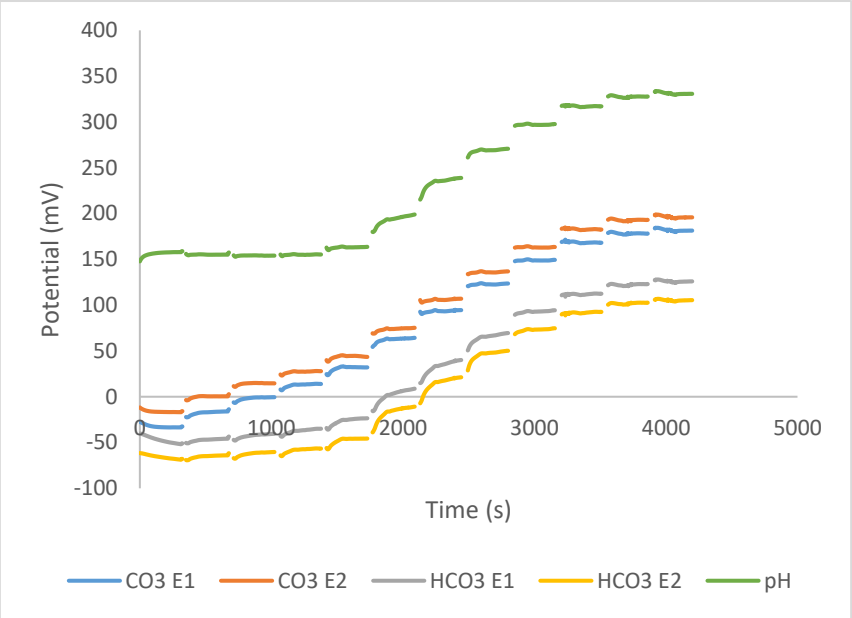


Figure 16: Solid-State Electrodes in Bicarbonate Solution. Time Response

Since the pH value plays a crucial role in carbonic acid equilibria, the electrodes were calibrated in Tris buffer (figure 17). Only one carbonate electrode and one bicarbonate electrode are shown in the figure for clarity. It is apparent that at higher carbonate concentrations, the buffer capacity is not strong enough to keep the [OH^-] constant. At lower carbonate concentrations, the [OH^-] remains constant and the interference from OH^- becomes significant. In bicarbonate solutions, however, [OH^-] remains fairly constant throughout the entire range of bicarbonate concentrations (figure 18).

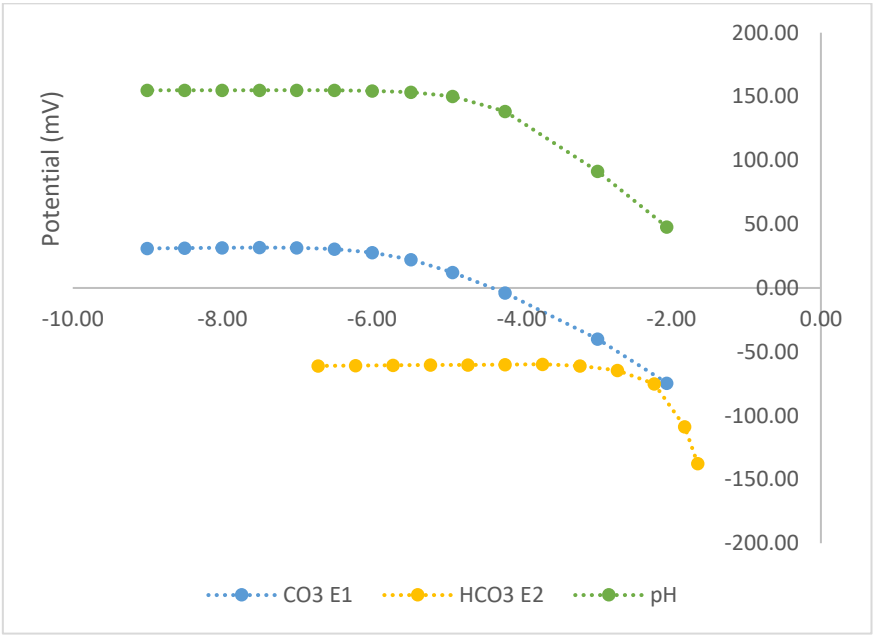


Figure 17: Solid State Electrodes in Carbonate Solution (Tris Buffer ~8.6 pH). The pH electrode was plotted against the logarithm of activity of carbonate

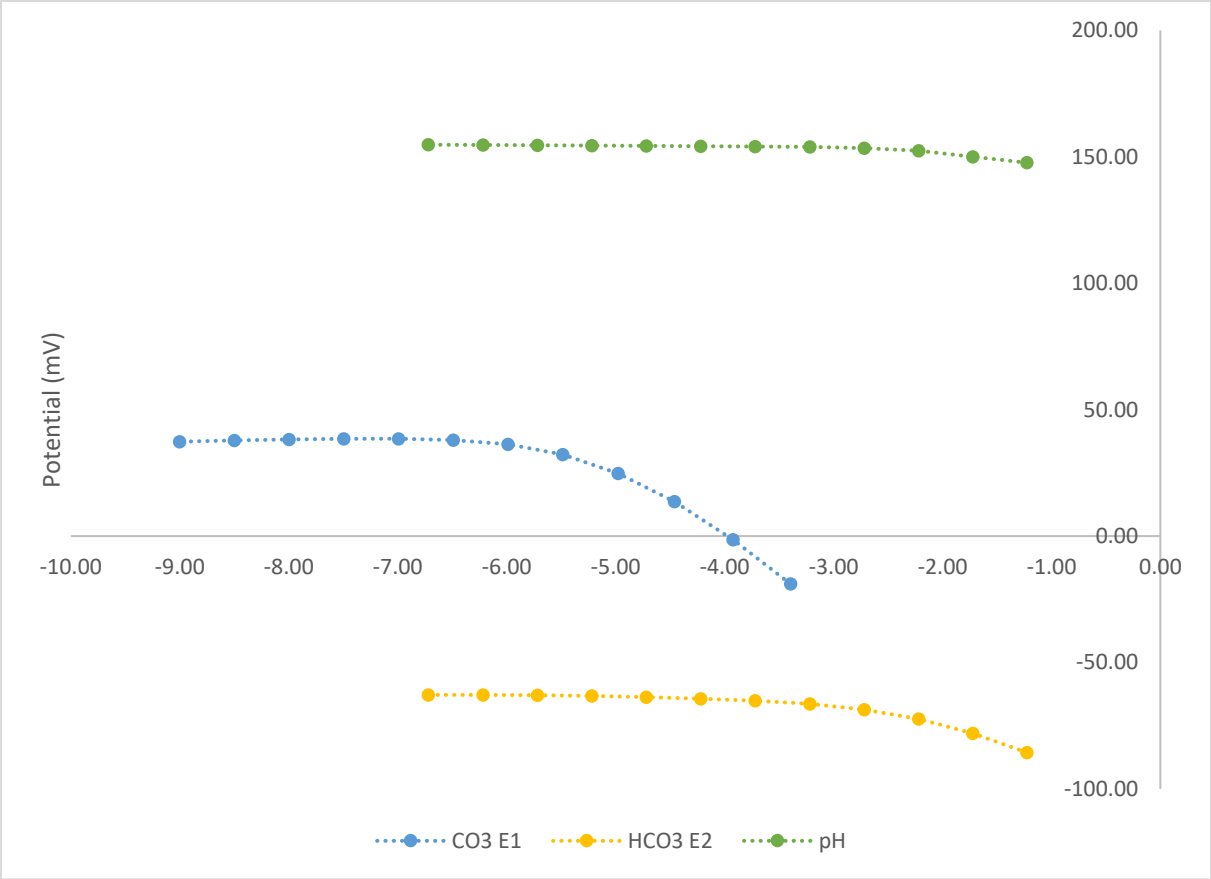


Figure 18: Solid State Electrodes in Bicarbonate Solution (Tris Buffer ~8.6 pH). The pH electrode was plotted against the logarithm of activity of bicarbonate

Imitating a closed system by using calibration solutions prepared using boiled water, and then preventing atmospheric CO_2 from dissolving in calibration solutions as much as possible would allow a more accurate determination of DIC.

Figures 19 & 20 illustrate the calibrations of electrodes in carbonate and bicarbonate solutions prepared using boiled water. The curves that were observed in earlier calibrations (near equivalence point) are much less prominent here. However, the slopes of the calibrations have also suffered an insignificant amount. The slopes from figure 19 are -28.3, -28.6, -57.4, and -57.8 mV/decade and the slopes from figure 20 are -22.9, -20.4, -29,6, and -30.5 mV/decade. Once again, considering the limited range from 10^{-3} M to 10^{-5} M, the slopes of the bicarbonate electrodes are improved to -47.7 and -48.5 mV/decade.

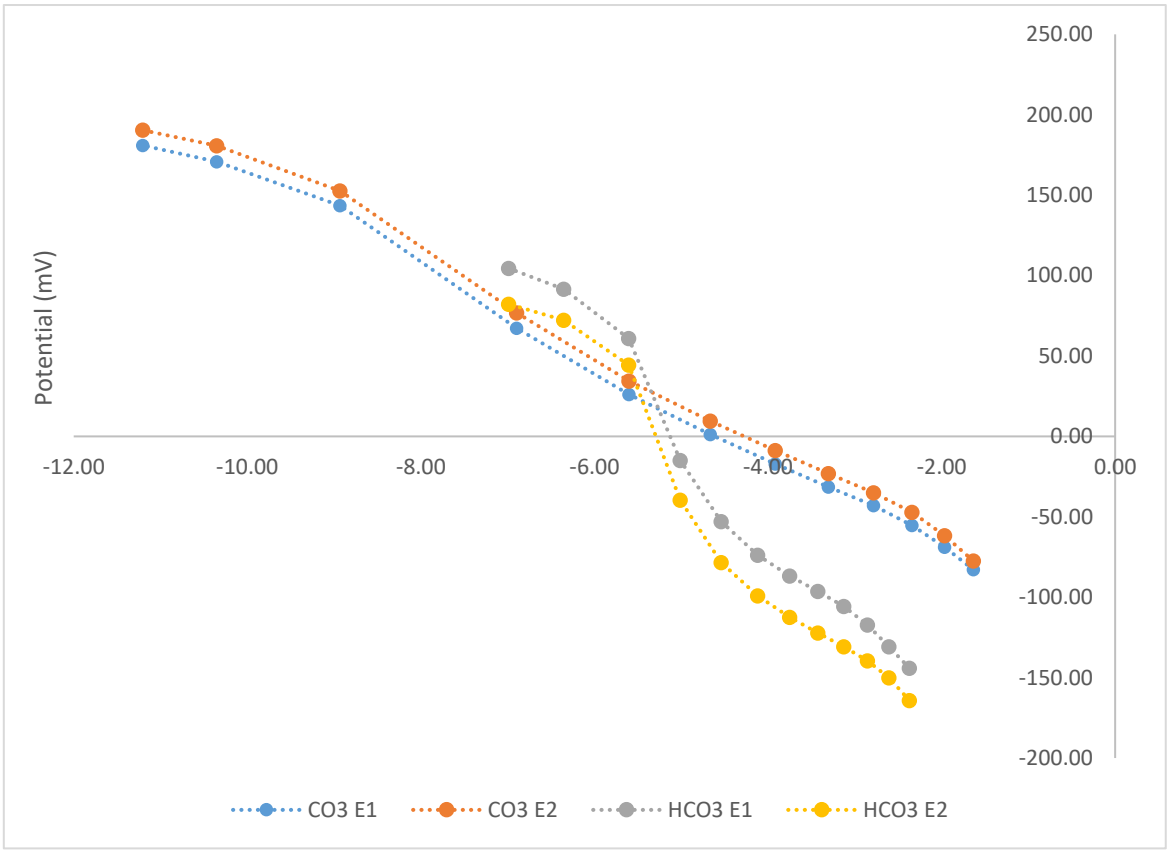


Figure 19: Solid-State Electrodes in Carbonate Solution (Prepared using Boiled Water) (pH range 11.6 – 6.1)

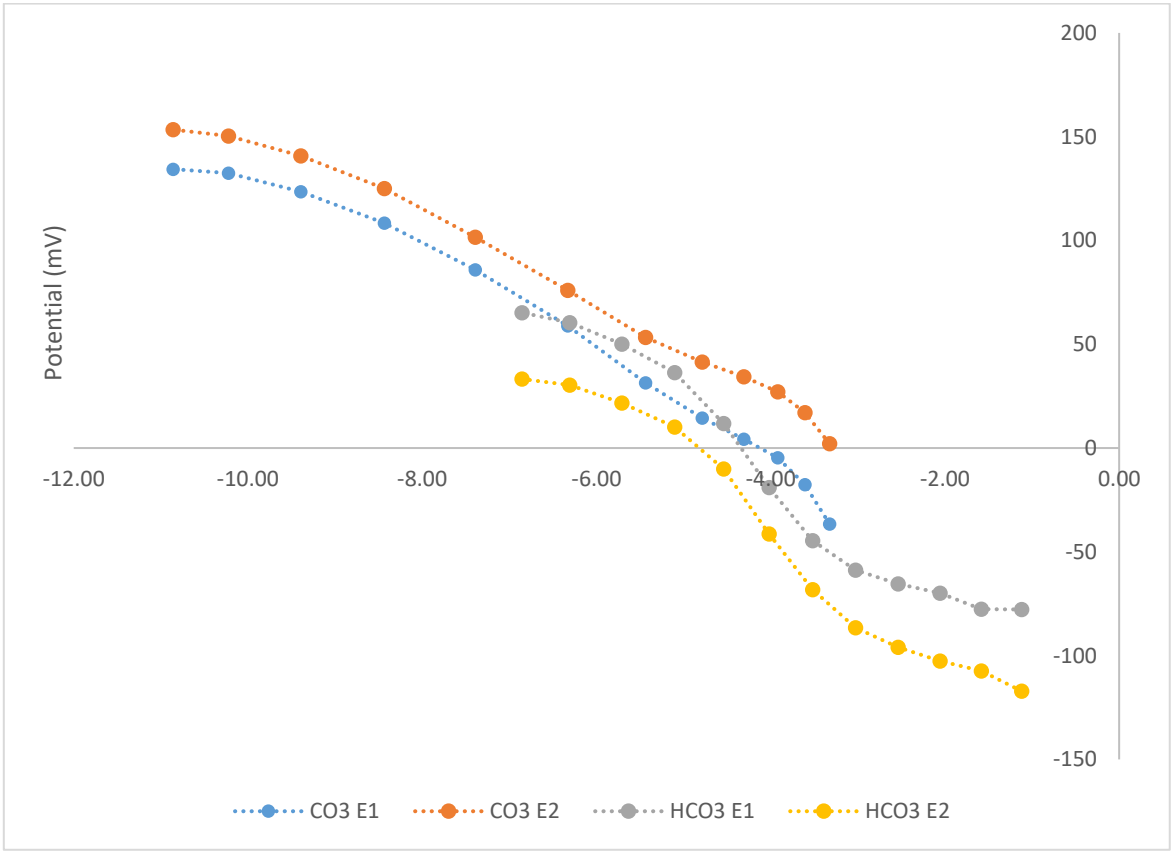


Figure 20: Solid-State Electrodes in Bicarbonate Solution (Prepared using Boiled Water) (pH range 8.4 – 6.3)

It can be seen from figure 21 that the drastic change in pH when the system is exposed to atmospheric air has been reduced when using calibration solutions prepared using boiled water imitating a pseudo closed system. However, the effect has not been eliminated yet as it is near impossible to emulate a perfectly closed system. The automatic dilution system has vents that can absorb atmospheric CO_2 in to the solutions. It would be worth investigating this behavior within a complete inert system.

Analogous behavior was observed, and comparable results were obtained using conventional carbonate and bicarbonate selective electrodes. Thus, it is evident that this behavior is a result of the chemistry of the system and it is independent of the type of contact used for ion-sensing.

A complete summary of calibrations can be found in Appendix A: table 19.

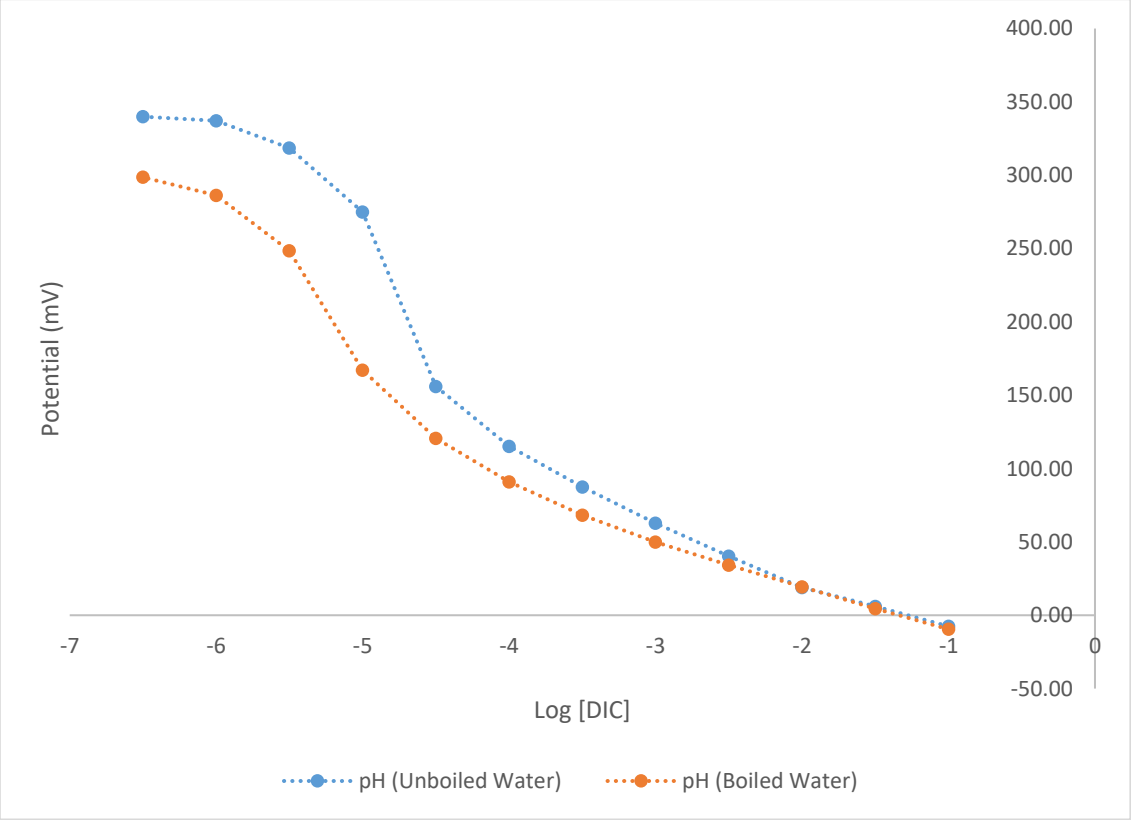


Figure 21: Comparison between pH measurements in solutions prepared using boiled and un-boiled water

4.3 Assessment of Selectivity Coefficients

Table 4: Carbonate Selectivity Coefficients for Solid-State Electrodes and Conventional Electrodes

	Solid State Electrodes		Conventional Electrodes	
	E1	E2	E1	E2
Log K $_{CO3^{2-},NO3^-}$	-2.9	-2.0	-1.7	-1.5
Log K $_{CO3^{2-},SO4^{2-}}$	-6.4	-6.5	-4.7	-4.7
Log K $_{CO3^{2-},Cl^-}$	-5.6	-5.7	-4.6	-4.6
Log K $_{CO3^{2-},Salicylate}$	0.7	0.7	2.5	2.4

Table 5: Bicarbonate Selectivity Coefficients for Solid-State Electrodes and Conventional Electrodes

	Solid State Electrodes		Conventional Electrodes	
	E1	E2	E1	E2
Log K $_{HCO3^-,NO3^-}$	-1.1	-1.5	-0.7	-0.8
Log K $_{HCO3^-,SO4^{2-}}$	-2.4	-2.7	-2.00	-2.3
Log K $_{HCO3^-,Cl^-}$	-1.6	-1.8	-1.4	-1.5
Log K $_{HCO3^-,Salicylate}$	0.1	-0.1	-0.05	-0.02

The selectivities were measured using the separate solution method at 10^{-2} M sodium salts of each analyte. In general, the solid-state electrodes (both carbonate and bicarbonate) perform better compared to conventional electrodes. With the exception of salicylate, the electrodes are capable of discriminating against other measured interfering ions. However, salicylate is known to cause selectivity issues due to its lipophilicity [23]. The salicylate is often present in blood of the patients who take anti-inflammatory medications like Aspirin.

4.4 Measurement of Sample

The pH electrode was recalibrated with a slope of -56.6 mV/decade and an intercept of 637.1 mV.

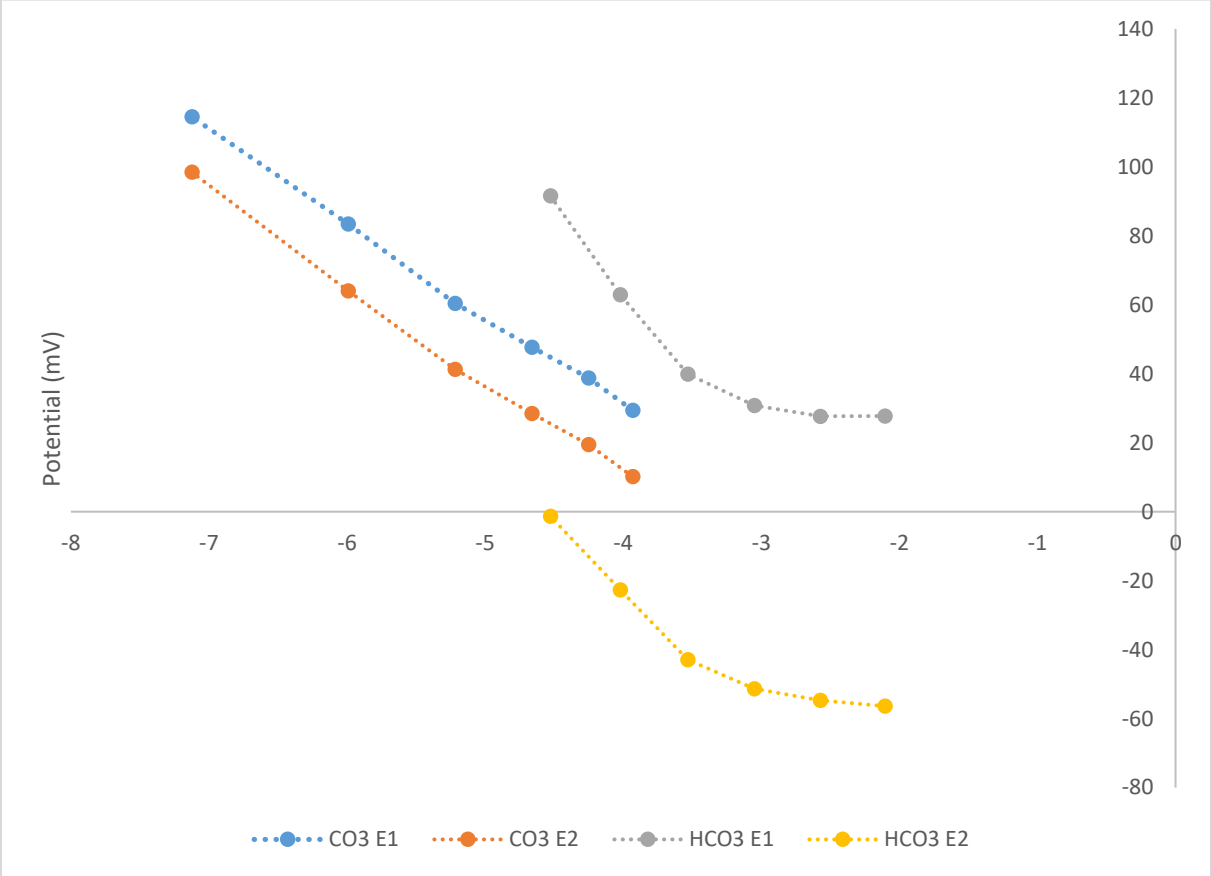


Figure 22: Calibration Curve before Sample Measurement

Table 6: Results from Sample Measurement

	Carbonate Electrodes		Bicarbonate Electrodes	
	E1	E2	E3	E4
Slope (mV/decade)	-26.5	-27.4	-25.8	-22.5
Intercept (mV)	-75.5	-98.6	-38.2	-112.6

It was determined that the sample contained 0.11 and 0.09 mmol/L of carbonate from carbonate electrodes and 14.7 and 13.7 mmol/L of bicarbonate from bicarbonate electrodes compared to the reference value of 36 mmol/L of bicarbonate. No reference value for carbonate was found. As observed earlier, the bicarbonate electrodes fail to respond in higher concentrations. This could very well be the reason for the difference in detected concentrations of bicarbonate as they fall outside of the detection limits.

Chapter 5: Conclusion

It has been observed that the carbonate electrodes fabricated for this thesis exhibit an excellent linear range of nearly 10 orders of magnitude with near ideal Nernstian slopes. The bicarbonate electrodes also show very promising results although the membrane composition may have to be further optimized to be able to detect higher concentrations of bicarbonate. Both carbonate and bicarbonate electrodes also exhibit reasonable selectivities. However, it would be worth remeasuring the selectivities using a different method to determine them more accurately.

It must be noted that all the data for this thesis were collected approximately four months after the fabrication of electrodes. Thus, the performance of the electrodes may have been deteriorated over the course of time due to leaching out of membrane components. Nonetheless, the electrodes still provide an excellent platform to characterize and comprehend the complex equilibria of carbonic acid species.

The measurement of the sample was unfortunately not very successful. The concentration of the sample falls outside of the detection limits of the electrodes. Sample was not diluted as it may change matrix properties. One interesting aspect that was observed during the sample measurement is that the sample also had a pH of 8.53 compared to the regular blood pH of 7.4. A reference value for pH was not provided by the manufacturer. Nonetheless, bicarbonate remains the dominant species in both pH values.

It is also evident that measurement of pH of the solutions is very decisive for accurate determination of carbonic acid species given the equilibria's high dependency on pH. In this thesis, the effect of pCO_2 has not been considered. Instead, an attempt was made to eliminate the said effect by emulating a closed system. Since current clinical method of determining serum bicarbonate levels already includes a pCO_2 electrode, it would be worth recalibrating the fabricated electrodes while measuring the pressure as well. To extend the application of the electrodes to environmental samples, especially in determination of carbonic acid species in seawater, the effects of atmospheric pressure and the temperature need to be considered.

In summary, this comprehensive study on carbonic acid species has found the fabricated electrodes to be very promising although much work remains to be done. Further research will be carried out, after the submission of this thesis, on improving selectivities as well as detection limits.

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Appendix A: Data

Table 7: Conventional Electrodes in Carbonate. All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	4.26	2.15	89.63	100.73	-7.35	11.52	11.64	0.12	6.07E-03	9.39E-02	-2.30	-1.64
-1.5	3.16E-02	20.53	18.44	100.20	102.68	7.83	11.25	11.38	0.13	3.39E-03	2.82E-02	-2.53	-1.97
-2	1.00E-02	34.88	33.33	108.64	105.41	22.25	10.99	11.12	0.13	1.78E-03	8.22E-03	-2.78	-2.35
-2.5	3.16E-03	47.04	46.27	112.13	105.64	35.71	10.75	10.85	0.10	8.64E-04	2.30E-03	-3.08	-2.80
-3	1.00E-03	60.56	60.58	117.09	108.82	51.38	10.48	10.55	0.07	4.17E-04	5.83E-04	-3.39	-3.32
-3.5	3.16E-04	77.73	77.88	124.51	116.04	71.08	10.13	10.23	0.10	1.94E-04	1.22E-04	-3.72	-3.96
-4	1.00E-04	97.02	97.93	133.49	125.38	92.72	9.74	9.87	0.13	7.95E-05	2.05E-05	-4.10	-4.71
-4.5	3.16E-05	130.40	130.74	155.76	147.08	127.94	9.12	9.44	0.32	2.98E-05	1.82E-06	-4.53	-5.75
-5	1.00E-05	206.03	198.81	222.01	202.83	228.99	7.32	8.98	1.66	9.01E-06	8.81E-09	-5.05	-8.06
-5.5	3.16E-06	244.61	237.77	256.45	246.25	288.45	6.26	8.49	2.23	1.41E-06	1.21E-10	-5.85	-9.92
-6	1.00E-06	269.70	265.38	271.29	271.91	311.69	5.85	8.01	2.16	2.37E-07	7.86E-12	-6.63	-11.11
-6.5	3.16E-07	278.73	275.35	278.39	287.19	319.46	5.71	7.56	1.85	5.82E-08	1.41E-12	-7.24	-11.85

Table 8: Conventional Electrodes in Bicarbonate. All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	76.58	75.11	109.38	125.84	173.22	8.31	8.35	0.04	9.80E-02	9.39E-04	-1.12	-3.47
-1.5	3.16E-02	91.92	89.48	118.17	132.87	169.72	8.37	8.35	-0.02	3.10E-02	3.43E-04	-1.58	-3.75
-2	1.00E-02	107.85	104.41	130.51	142.99	166.98	8.42	8.35	-0.08	9.79E-03	1.21E-04	-2.05	-4.10
-2.5	3.16E-03	128.09	124.17	146.27	155.36	167.64	8.41	8.34	-0.07	3.10E-03	3.73E-05	-2.54	-4.54
-3	1.00E-03	154.02	153.02	168.74	175.13	175.91	8.26	8.31	0.05	9.79E-04	8.41E-06	-3.02	-5.14

-3.5	3.16E-04	181.59	180.65	197.68	201.55	202.10	7.80	8.24	0.44	3.04E-04	8.95E-07	-3.53	-6.08
-4	1.00E-04	208.18	206.82	224.18	225.91	235.29	7.21	8.11	0.90	8.75E-05	6.62E-08	-4.06	-7.20
-4.5	3.16E-05	233.32	233.35	250.13	250.81	265.43	6.67	7.91	1.24	2.13E-05	4.69E-09	-4.67	-8.34
-5	1.00E-05	255.27	254.42	270.69	271.78	291.76	6.21	7.69	1.48	4.12E-06	3.09E-10	-5.39	-9.52
-5.5	3.16E-06	269.60	266.69	285.28	288.58	312.15	5.84	7.46	1.61	7.38E-07	2.40E-11	-6.13	-10.62
-6	1.00E-06	277.62	273.93	296.06	301.65	323.61	5.64	7.25	1.61	1.60E-07	3.26E-12	-6.80	-11.49
-6.5	3.16E-07	281.44	277.46	302.74	309.76	327.65	5.57	7.11	1.54	4.39E-08	7.60E-13	-7.36	-12.12

Table 9: Solid-State Electrodes in Carbonate. All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-123.67	-109.93	-121.02	-158.17	-7.54	11.52	11.64	0.12	6.02E-03	9.40E-02	-2.31	-1.64
-1.5	3.16E-02	-108.59	-94.86	-109.60	-146.00	6.12	11.28	11.38	0.10	3.19E-03	2.84E-02	-2.55	-1.97
-2	1.00E-02	-95.10	-81.47	-101.79	-137.06	18.87	11.05	11.12	0.07	1.59E-03	8.41E-03	-2.83	-2.34
-2.5	3.16E-03	-72.87	-60.33	-88.67	-122.39	40.32	10.67	10.85	0.18	9.87E-04	2.17E-03	-3.03	-2.82
-3	1.00E-03	-54.05	-39.60	-74.69	-107.54	62.71	10.28	10.55	0.27	5.32E-04	4.68E-04	-3.29	-3.41
-3.5	3.16E-04	-31.08	-16.70	-60.12	-91.52	87.48	9.83	10.23	0.40	2.40E-04	7.66E-05	-3.63	-4.16
-4	1.00E-04	-5.19	10.11	-44.05	-73.19	115.20	9.34	9.87	0.53	9.06E-05	9.32E-06	-4.05	-5.05
-4.5	3.16E-05	36.65	50.54	-13.88	-39.02	156.06	8.62	9.44	0.82	3.09E-05	5.97E-07	-4.51	-6.24
-5	1.00E-05	130.54	142.24	79.36	63.30	274.91	6.50	8.98	2.48	5.83E-06	8.72E-10	-5.24	-9.07
-5.5	3.16E-06	167.81	180.93	129.61	105.40	318.59	5.73	8.49	2.76	5.99E-07	1.50E-11	-6.22	-10.83
-6	1.00E-06	188.19	203.31	145.99	121.73	337.14	5.40	8.01	2.61	9.86E-08	1.16E-12	-7.01	-11.94
-6.5	3.16E-07	190.90	205.32	147.18	122.84	339.91	5.35	7.56	2.21	2.82E-08	2.95E-13	-7.55	-12.53

Table 10: Solid-State Electrodes in Bicarbonate. All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-32.68	-16.02	-51.13	-68.06	158.72	8.57	8.35	-0.22	9.77E-02	1.69E-03	-1.12	-3.21
-1.5	3.16E-02	-14.62	1.85	-44.35	-62.52	156.62	8.61	8.35	-0.26	3.09E-02	5.83E-04	-1.58	-3.52
-2	1.00E-02	-0.53	14.60	-40.48	-60.38	154.04	8.65	8.35	-0.31	9.75E-03	2.05E-04	-2.06	-3.87
-2.5	3.16E-03	13.92	27.89	-34.96	-56.65	155.29	8.63	8.34	-0.29	3.08E-03	6.16E-05	-2.54	-4.32
-3	1.00E-03	32.01	43.47	-23.57	-45.69	163.57	8.48	8.31	-0.17	9.79E-04	1.39E-05	-3.02	-4.92
-3.5	3.16E-04	64.24	75.20	8.65	-10.93	198.81	7.86	8.24	0.39	3.05E-04	1.03E-06	-3.52	-6.02
-4	1.00E-04	94.55	106.98	40.00	21.16	238.83	7.15	8.11	0.96	8.59E-05	5.62E-08	-4.07	-7.27
-4.5	3.16E-05	123.56	136.82	69.45	50.12	270.70	6.58	7.91	1.33	1.97E-05	3.51E-09	-4.71	-8.47
-5	1.00E-05	149.42	163.47	94.37	74.70	297.66	6.10	7.69	1.59	3.55E-06	2.09E-10	-5.45	-9.69
-5.5	3.16E-06	168.13	182.53	112.44	92.61	317.05	5.76	7.46	1.70	6.30E-07	1.68E-11	-6.20	-10.78
-6	1.00E-06	178.12	193.08	123.00	102.67	327.75	5.57	7.25	1.69	1.38E-07	2.38E-12	-6.86	-11.62
-6.5	3.16E-07	181.34	195.82	125.92	105.43	330.69	5.51	7.11	1.60	3.94E-08	6.02E-13	-7.40	-12.22

Table 11: Conventional Electrodes in Carbonate (Tris Buffer ~ 8.6 pH). All Concentrations are in mol/L

		Carbonate		Bicarbonate				Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	34.00	31.61	112.77	113.14	67.72	10.19	5.82E-02	4.18E-02	-1.45	-2.24
-1.5	3.16E-02	72.31	69.61	141.37	138.82	131.72	9.05	3.00E-02	1.57E-03	-1.73	-3.64
-2	1.00E-02	100.87	98.22	158.66	157.88	161.31	8.52	9.78E-03	1.53E-04	-2.22	-4.65
-2.5	3.16E-03	117.86	114.94	162.55	163.90	168.59	8.39	3.10E-03	3.59E-05	-2.72	-5.28
-3	1.00E-03	131.08	128.40	164.43	167.15	171.17	8.35	9.80E-04	1.02E-05	-3.22	-5.82
-3.5	3.16E-04	140.38	138.03	165.35	169.12	171.86	8.34	3.10E-04	3.14E-06	-3.72	-6.33
-4	1.00E-04	145.83	144.13	166.01	170.51	171.95	8.33	9.80E-05	9.89E-07	-4.22	-6.83

-4.5	3.16E-05	148.79	147.48	166.89	171.84	172.02	8.33	3.10E-05	3.12E-07	-4.72	-7.34
-5	1.00E-05	150.37	149.13	167.87	173.13	172.09	8.33	9.80E-06	9.83E-08	-5.22	-7.84
-5.5	3.16E-06	151.25	150.01	168.97	174.31	172.19	8.33	3.10E-06	3.10E-08	-5.72	-8.34
-6	1.00E-06	152.03	150.73	170.29	175.67	172.51	8.32	9.80E-07	9.67E-09	-6.22	-8.84
-6.5	3.16E-07	152.15	150.85	171.16	176.52	172.50	8.32	3.10E-07	3.06E-09	-6.72	-9.34

Table 12: Conventional Electrodes in Bicarbonate (Tris Buffer ~ 8.6 pH). All Concentrations are in mol/L

		Carbonate		Bicarbonate				Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	69.60	68.48	103.25	113.14	161.09	8.53	9.78E-02	1.54E-03	-1.22	-3.66
-1.5	3.16E-02	89.84	87.84	119.98	128.32	167.99	8.40	3.10E-02	3.68E-04	-1.72	-4.27
-2	1.00E-02	104.46	101.99	132.79	138.44	169.80	8.37	9.80E-03	1.08E-04	-2.22	-4.80
-2.5	3.16E-03	117.49	114.68	142.96	146.39	170.39	8.36	3.10E-03	3.33E-05	-2.72	-5.31
-3	1.00E-03	128.68	125.02	150.57	152.38	170.65	8.36	9.80E-04	1.04E-05	-3.22	-5.81
-3.5	3.16E-04	137.18	133.66	156.14	157.04	170.76	8.36	3.10E-04	3.28E-06	-3.72	-6.31
-4	1.00E-04	143.02	139.42	160.33	160.63	170.78	8.35	9.80E-05	1.04E-06	-4.22	-6.81
-4.5	3.16E-05	146.47	143.19	163.67	163.58	170.85	8.35	3.10E-05	3.27E-07	-4.72	-7.31
-5	1.00E-05	148.43	145.52	166.38	166.06	170.88	8.35	9.80E-06	1.03E-07	-5.22	-7.82
-5.5	3.16E-06	149.69	146.96	168.66	168.08	170.91	8.35	3.10E-06	3.26E-08	-5.72	-8.32
-6	1.00E-06	150.49	147.97	170.53	169.79	170.88	8.35	9.80E-07	1.03E-08	-6.22	-8.82
-6.5	3.16E-07	151.12	148.75	172.15	171.29	170.92	8.35	3.10E-07	3.26E-09	-6.72	-9.32

Table 13: Solid-State Electrodes in Carbonate (Tris Buffer ~ 8.6 pH). All Concentrations are in mol/L

		Carbonate		Bicarbonate				Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-74.49	-66.92	-89.46	-137.66	47.83	10.54	3.82E-02	6.18E-02	-1.63	-2.07
-1.5	3.16E-02	-40.00	-30.42	-69.65	-108.65	91.44	9.76	2.49E-02	6.76E-03	-1.81	-3.01
-2	1.00E-02	-3.86	6.65	-43.88	-75.08	138.41	8.93	9.59E-03	3.82E-04	-2.23	-4.25
-2.5	3.16E-03	12.25	22.51	-36.46	-64.46	150.05	8.72	3.07E-03	7.60E-05	-2.72	-4.95
-3	1.00E-03	22.12	31.97	-34.06	-60.98	153.41	8.66	9.74E-04	2.10E-05	-3.22	-5.51
-3.5	3.16E-04	27.72	37.24	-33.52	-59.74	154.45	8.65	3.08E-04	6.37E-06	-3.72	-6.03
-4	1.00E-04	30.38	39.73	-33.65	-60.13	154.82	8.64	9.75E-05	1.98E-06	-4.22	-6.53
-4.5	3.16E-05	31.44	40.64	-34.38	-60.23	154.99	8.64	3.08E-05	6.23E-07	-4.72	-7.04
-5	1.00E-05	31.64	40.67	-35.08	-60.31	154.89	8.64	9.75E-06	1.98E-07	-5.22	-7.53
-5.5	3.16E-06	31.52	40.47	-35.85	-60.57	154.87	8.64	3.08E-06	6.26E-08	-5.72	-8.03
-6	1.00E-06	31.33	40.20	-36.54	-60.94	154.88	8.64	9.75E-07	1.98E-08	-6.22	-8.53
-6.5	3.16E-07	31.07	39.92	-37.37	-61.10	154.89	8.64	3.08E-07	6.26E-09	-6.72	-9.03

Table 14: Solid-State Electrodes in Bicarbonate (Tris Buffer ~ 8.6 pH). All Concentrations are in mol/L

		Carbonate		Bicarbonate				Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-18.90	-7.58	-60.68	-85.56	147.68	8.77	9.70E-02	2.64E-03	-1.22	-3.42
-1.5	3.16E-02	-1.44	10.30	-51.68	-77.97	149.96	8.72	3.07E-02	7.63E-04	-1.72	-3.95
-2	1.00E-02	13.65	24.47	-44.88	-72.26	152.39	8.68	9.73E-03	2.19E-04	-2.22	-4.49
-2.5	3.16E-03	24.79	34.45	-40.93	-68.57	153.42	8.66	3.08E-03	6.64E-05	-2.72	-5.01
-3	1.00E-03	32.26	40.38	-38.60	-66.37	153.91	8.65	9.74E-04	2.06E-05	-3.22	-5.52
-3.5	3.16E-04	36.27	43.28	-37.34	-65.13	154.07	8.65	3.08E-04	6.47E-06	-3.72	-6.02
-4	1.00E-04	37.94	44.35	-36.68	-64.31	154.17	8.65	9.75E-05	2.04E-06	-4.22	-6.52

-4.5	3.16E-05	38.52	44.55	-36.20	-63.65	154.36	8.65	3.08E-05	6.39E-07	-4.72	-7.02
-5	1.00E-05	38.44	44.32	-35.96	-63.23	154.45	8.65	9.75E-06	2.01E-07	-5.22	-7.53
-5.5	3.16E-06	38.23	44.09	-35.76	-62.90	154.62	8.64	3.08E-06	6.32E-08	-5.72	-8.03
-6	1.00E-06	37.83	43.82	-35.75	-62.79	154.72	8.64	9.75E-07	1.99E-08	-6.22	-8.53
-6.5	3.16E-07	37.39	43.46	-35.93	-62.80	154.76	8.64	3.08E-07	6.29E-09	-6.72	-9.03

Table 15: Conventional Electrodes in Carbonate (Boiled Water). All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	4.60	3.38	92.00	108.28	-10.01	11.57	11.64	0.07	5.48E-03	9.45E-02	-2.35	-1.63
-1.5	3.16E-02	19.81	17.64	103.47	114.63	4.28	11.31	11.38	0.07	2.98E-03	2.86E-02	-2.58	-1.96
-2	1.00E-02	35.74	33.64	116.45	117.60	20.28	11.03	11.12	0.09	1.67E-03	8.33E-03	-2.81	-2.35
-2.5	3.16E-03	50.45	48.87	123.05	120.95	35.97	10.75	10.85	0.10	8.71E-04	2.29E-03	-3.08	-2.80
-3	1.00E-03	65.86	64.68	129.33	124.63	52.69	10.45	10.55	0.10	4.30E-04	5.70E-04	-3.38	-3.33
-3.5	3.16E-04	82.46	81.51	134.36	129.67	70.58	10.14	10.23	0.09	1.93E-04	1.23E-04	-3.72	-3.96
-4	1.00E-04	103.25	102.59	143.24	138.35	92.06	9.75	9.87	0.12	7.90E-05	2.10E-05	-4.11	-4.70
-4.5	3.16E-05	129.93	129.83	157.17	152.10	117.57	9.30	9.44	0.14	2.89E-05	2.70E-06	-4.54	-5.58
-5	1.00E-05	168.85	171.68	181.29	175.06	150.60	8.71	8.98	0.27	9.72E-06	2.35E-07	-5.01	-6.64
-5.5	3.16E-06	223.21	225.06	229.89	218.38	206.04	7.73	8.49	0.76	3.03E-06	7.57E-09	-5.52	-8.12
-6	1.00E-06	253.97	252.04	270.69	255.76	261.96	6.73	8.01	1.28	7.03E-07	1.79E-10	-6.15	-9.75
-6.5	3.16E-07	269.68	266.52	282.15	271.64	284.37	6.34	7.56	1.22	1.54E-07	1.56E-11	-6.81	-10.81

Table 16: Conventional Electrodes in Bicarbonate (Boiled Water). All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	75.27	74.93	106.68	124.12	170.93	8.35	8.35	0.00	9.80E-02	1.03E-03	-1.12	-3.43
-1.5	3.16E-02	90.76	88.91	117.29	134.55	167.65	8.41	8.35	-0.06	3.10E-02	3.73E-04	-1.58	-3.72
-2	1.00E-02	106.34	103.66	129.78	143.55	163.94	8.48	8.35	-0.13	9.79E-03	1.37E-04	-2.05	-4.04
-2.5	3.16E-03	123.20	121.36	143.45	151.68	160.66	8.53	8.34	-0.20	3.09E-03	4.95E-05	-2.54	-4.41
-3	1.00E-03	146.59	146.24	158.70	165.39	161.99	8.51	8.31	-0.20	9.78E-04	1.48E-05	-3.02	-4.89
-3.5	3.16E-04	173.46	174.06	180.70	184.89	171.96	8.33	8.24	-0.09	3.10E-04	3.13E-06	-3.52	-5.54
-4	1.00E-04	200.78	201.02	205.44	207.52	195.10	7.92	8.11	0.19	9.70E-05	3.80E-07	-4.02	-6.44
-4.5	3.16E-05	225.84	225.98	232.06	230.26	225.87	7.38	7.91	0.54	2.88E-05	3.21E-08	-4.54	-7.51
-5	1.00E-05	245.90	245.49	251.39	248.53	254.15	6.87	7.69	0.81	7.65E-06	2.68E-09	-5.12	-8.58
-5.5	3.16E-06	258.13	256.89	264.36	262.59	276.74	6.47	7.46	0.98	1.78E-06	2.48E-10	-5.75	-9.61
-6	1.00E-06	264.62	263.02	273.55	273.09	289.72	6.24	7.25	1.01	4.32E-07	3.53E-11	-6.36	-10.45
-6.5	3.16E-07	267.05	265.31	277.77	279.58	294.83	6.15	7.11	0.96	1.21E-07	8.00E-12	-6.92	-11.10

Table 17: Solid-State Electrodes in Carbonate (Boiled Water). All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-82.86	-77.46	-144.16	-164.24	-9.54	11.56	11.64	0.08	5.58E-03	9.44E-02	-2.34	-1.63
-1.5	3.16E-02	-68.82	-61.62	-130.87	-150.16	4.50	11.31	11.38	0.07	3.00E-03	2.86E-02	-2.58	-1.96
-2	1.00E-02	-55.30	-47.13	-117.25	-139.52	19.26	11.05	11.12	0.07	1.61E-03	8.39E-03	-2.83	-2.34
-2.5	3.16E-03	-43.07	-34.95	-105.74	-130.83	34.12	10.78	10.85	0.07	8.24E-04	2.34E-03	-3.11	-2.79
-3	1.00E-03	-31.42	-23.02	-96.42	-122.17	49.97	10.50	10.55	0.05	4.03E-04	5.97E-04	-3.41	-3.31
-3.5	3.16E-04	-17.04	-8.85	-86.82	-112.53	68.33	10.18	10.23	0.05	1.86E-04	1.30E-04	-3.74	-3.93

-4	1.00E-04	1.23	9.46	-73.83	-99.09	90.89	9.77	9.87	0.10	7.82E-05	2.18E-05	-4.11	-4.69
-4.5	3.16E-05	26.05	34.49	-52.99	-78.45	120.55	9.25	9.44	0.19	2.92E-05	2.41E-06	-4.54	-5.63
-5	1.00E-05	67.20	76.78	-15.00	-39.59	167.08	8.42	8.98	0.56	9.79E-06	1.21E-07	-5.01	-6.92
-5.5	3.16E-06	143.50	152.76	60.86	44.46	248.54	6.97	8.49	1.52	2.54E-06	1.12E-09	-5.60	-8.95
-6	1.00E-06	170.74	180.83	91.49	72.35	286.26	6.30	8.01	1.71	4.67E-07	4.39E-11	-6.33	-10.36
-6.5	3.16E-07	180.95	190.41	104.50	82.26	298.63	6.08	7.56	1.48	1.09E-07	6.20E-12	-6.96	-11.21

Table 18: Solid-State Electrodes in Bicarbonate (Boiled Water). All concentrations are in mol/L

		Carbonate		Bicarbonate						Recalculated from equilibria and pH			
Log [DIC]	[DIC]	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH (measured)	pH (predicted)	Δ pH	$[HCO_3^-]$	$[CO_3^{2-}]$	Log a HCO_3^-	Log a CO_3^{2-}
-1	1.00E-01	-36.6134	2.09972	-77.7825	-117.105	166.6168	8.43	8.35	-0.08	9.79E-02	1.23E-03	-1.12	-3.35
-1.5	3.16E-02	-17.5462	17.16774	-77.5765	-107.388	163.0488	8.49	8.35	-0.14	3.09E-02	4.50E-04	-1.58	-3.64
-2	1.00E-02	-4.71436	27.19452	-69.8296	-102.639	158.6669	8.57	8.35	-0.22	9.77E-03	1.70E-04	-2.05	-3.95
-2.5	3.16E-03	4.34078	34.31452	-65.4874	-96.0067	156.4879	8.61	8.34	-0.27	3.09E-03	5.87E-05	-2.54	-4.34
-3	1.00E-03	14.54852	41.45296	-58.8035	-86.5341	157.7839	8.59	8.31	-0.28	9.77E-04	1.76E-05	-3.03	-4.82
-3.5	3.16E-04	31.48484	53.28734	-44.5354	-68.1277	167.7198	8.41	8.24	-0.17	3.10E-04	3.72E-06	-3.52	-5.47
-4	1.00E-04	58.83956	76.08996	-18.953	-41.3137	190.4838	8.00	8.11	0.10	9.73E-05	4.60E-07	-4.02	-6.36
-4.5	3.16E-05	85.93714	101.6265	11.90606	-10.0402	221.411	7.46	7.91	0.46	2.92E-05	3.90E-08	-4.54	-7.42
-5	1.00E-05	108.3906	125.0588	36.31154	10.14634	248.5124	6.97	7.69	0.71	8.04E-06	3.54E-09	-5.10	-8.46
-5.5	3.16E-06	123.5607	140.7957	50.0291	21.70694	268.7154	6.61	7.46	0.84	2.03E-06	3.92E-10	-5.69	-9.41
-6	1.00E-06	132.3871	150.2778	60.38646	30.41842	281.561	6.39	7.25	0.87	5.15E-07	5.87E-11	-6.29	-10.23
-6.5	3.16E-07	134.3789	153.4119	65.25246	33.26662	286.5777	6.30	7.11	0.81	1.47E-07	1.36E-11	-6.83	-10.87

Table 19: Calibration Summary. Slope in mV/decade and Intercept in mV

Calibration	Carbonate Electrodes				Bicarbonate Electrodes			
	E1		E2		E3		E4	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Solid-State Electrodes in Carbonate	-29.57	-158.48	-29.58	-144.75	-57.93	-262.58	-60.79	-304.24
Solid-State Electrodes in Bicarbonate	-23.67	-89.28	-23.61	-74.86	-32.88	-101.80	-32.67	-120.67
Conventional Electrodes in Carbonate	-27.26	-30.28	-26.79	-29.92	-42.90	-19.74	-42.71	-23.07
Conventional Electrodes in Bicarbonate	-23.41	20.75	-23.32	19.19	-34.27	70.10	-32.55	83.91
Solid-State Electrodes in Carbonate (boiled water)	-28.30	-125.71	-28.60	-118.92	-57.38	-289.86	-57.83	-314.34
Solid-State Electrodes in Bicarbonate (boiled water)	-22.85	-95.65	-20.36	-55.51	-29.63	-130.25	-30.54	-162.47
Conventional Electrodes in Carbonate (boiled water)	-29.71	-34.85	-29.78	-36.05	-43.29	-17.69	-38.10	1.02
Conventional Electrodes in Bicarbonate (boiled water)	-25.17	14.88	-25.16	14.01	-32.99	66.91	-29.63	85.36

Table 20: Calibration Data for Sample Measurement

Log [DIC]	Log a HCO_3^-	Log a CO_3^{2-}	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH
-2	-2.10	-3.93	29.41	10.17	27.79	-56.38	137.92	8.82
-2.5	-2.57	-4.25	38.75	19.47	27.69	-54.69	136.36	8.85
-3	-3.05	-4.66	47.66	28.45	30.84	-51.39	137.45	8.83
-3.5	-3.53	-5.22	60.41	41.25	39.88	-42.89	144.86	8.70
-4	-4.02	-5.99	83.45	64.06	62.96	-22.68	163.16	8.38
-4.5	-4.53	-7.12	114.50	98.45	91.59	-1.35	199.69	7.73
Abtrol Sample			28.96	11.60	8.93	-70.64	154.81	8.53

Table 21: Selectivity Measurements for Conventional Electrodes Starting with Carbonate

	Carbonate		Bicarbonate		pH	
	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH
Carbonate	27.81	23.45	103.40	102.31	9.19	11.23
Nitrate	142.95	133.91	174.21	172.06	189.44	8.02
Sulphate	167.27	162.80	198.27	231.20	283.46	6.35
Chloride	228.26	223.58	245.11	254.04	286.02	6.31
Salicylate	19.58	17.19	157.95	182.02	286.14	6.31

Table 22: Selectivity Measurements for Conventional Electrodes Starting with Bicarbonate

	Carbonate		Bicarbonate		pH	
	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH
Bicarbonate	93.19	92.69	194.64	195.53	158.49	8.57
Nitrate	149.90	150.48	233.97	239.75	265.38	6.67
Sulphate	168.33	164.18	252.20	270.00	286.71	6.30
Chloride	228.95	227.81	275.42	283.84	291.66	6.21
Salicylate	18.62	16.48	197.31	196.96	288.78	6.26

Table 23: Selectivity Measurements for Solid-State Electrodes Starting with Carbonate

	Carbonate		Bicarbonate		pH	
	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH
Carbonate	-66.02	-65.02	-71.60	-131.09	11.40	11.19
Nitrate	84.77	58.83	53.35	4.26	122.94	9.20
Sulphate	123.13	126.90	80.41	29.56	302.97	6.01
Chloride	165.10	168.46	85.62	45.13	310.66	5.87
Salicylate	-22.55	-21.45	-20.23	-67.22	296.60	6.12

Table 24: Selectivity Measurements for Solid-State Electrodes Starting with Bicarbonate

	Carbonate		Bicarbonate		pH	
	E1 (mV)	E2 (mV)	E3 (mV)	E4 (mV)	pH (mV)	pH
Bicarbonate	24.43	30.94	-14.18	-78.11	159.74	8.55
Nitrate	95.99	97.03	51.63	7.35	283.97	6.34
Sulphate	121.89	126.51	69.16	17.95	298.16	6.09
Chloride	163.04	168.70	77.31	27.91	313.01	5.83
Salicylate	-24.87	-22.04	-22.28	-69.61	298.12	6.09

Appendix B: Figures Not Included in the Discussion

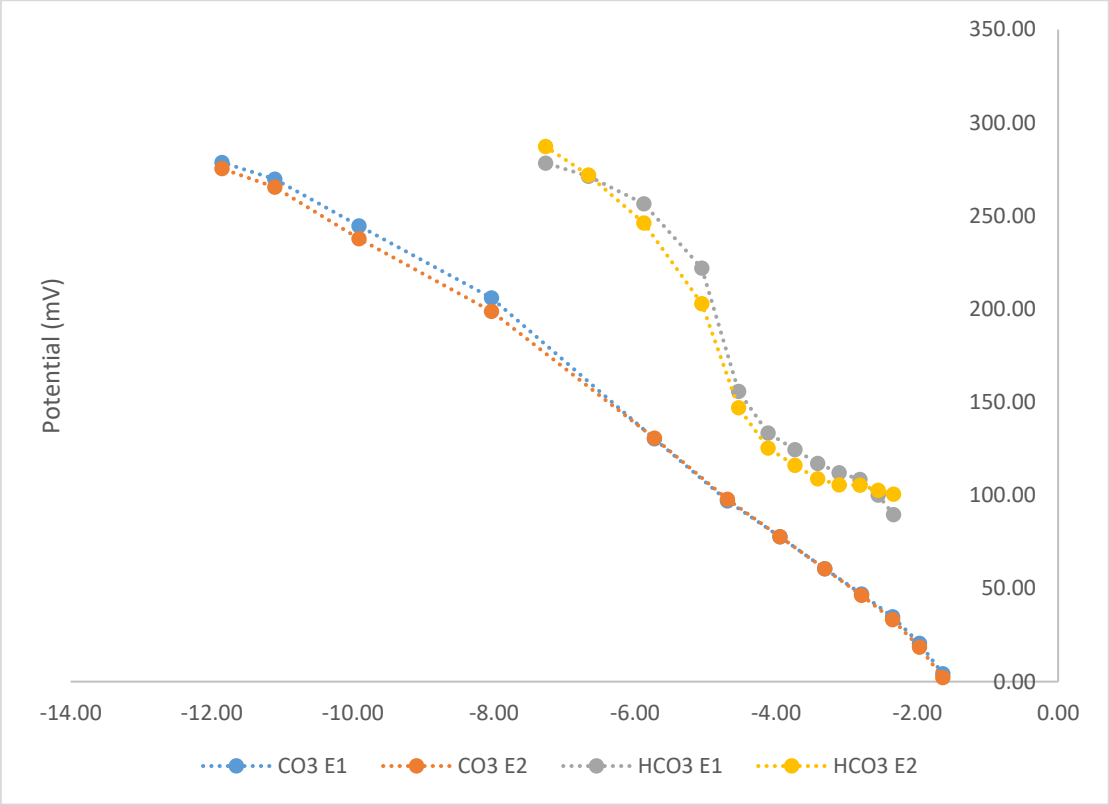

Figure 23: Conventional Electrodes in Carbonate

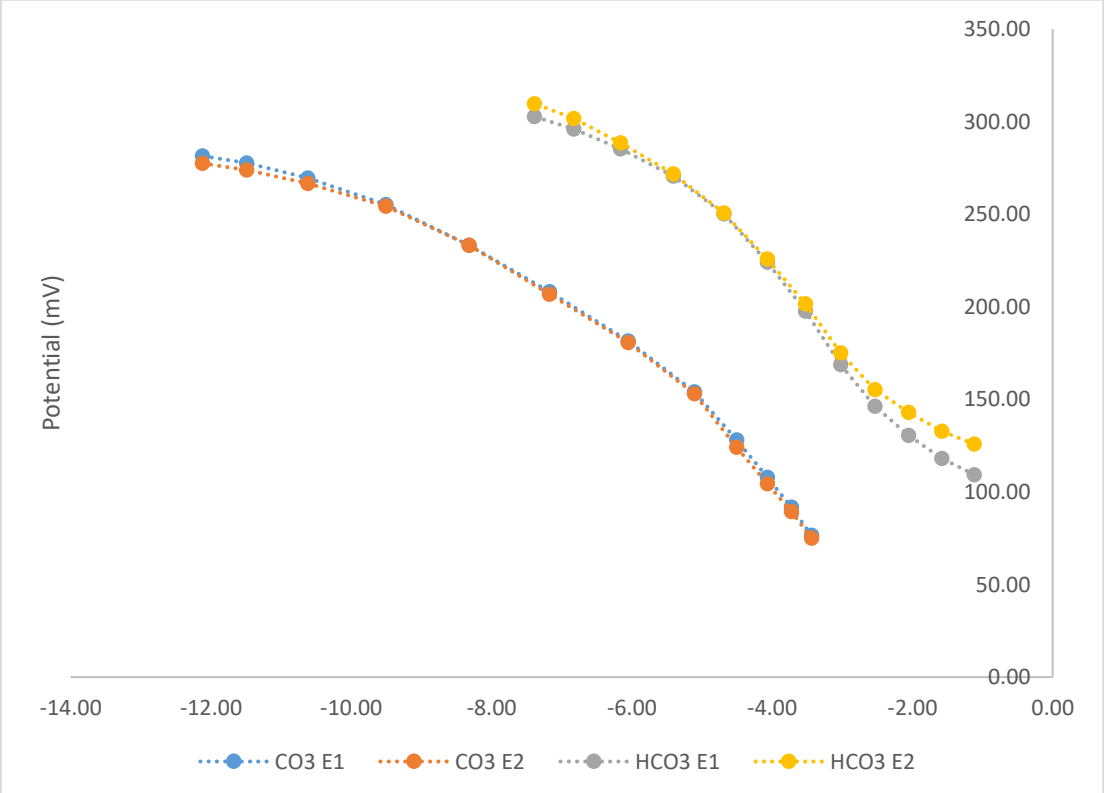

Figure 24: Conventional Electrodes in Bicarbonate

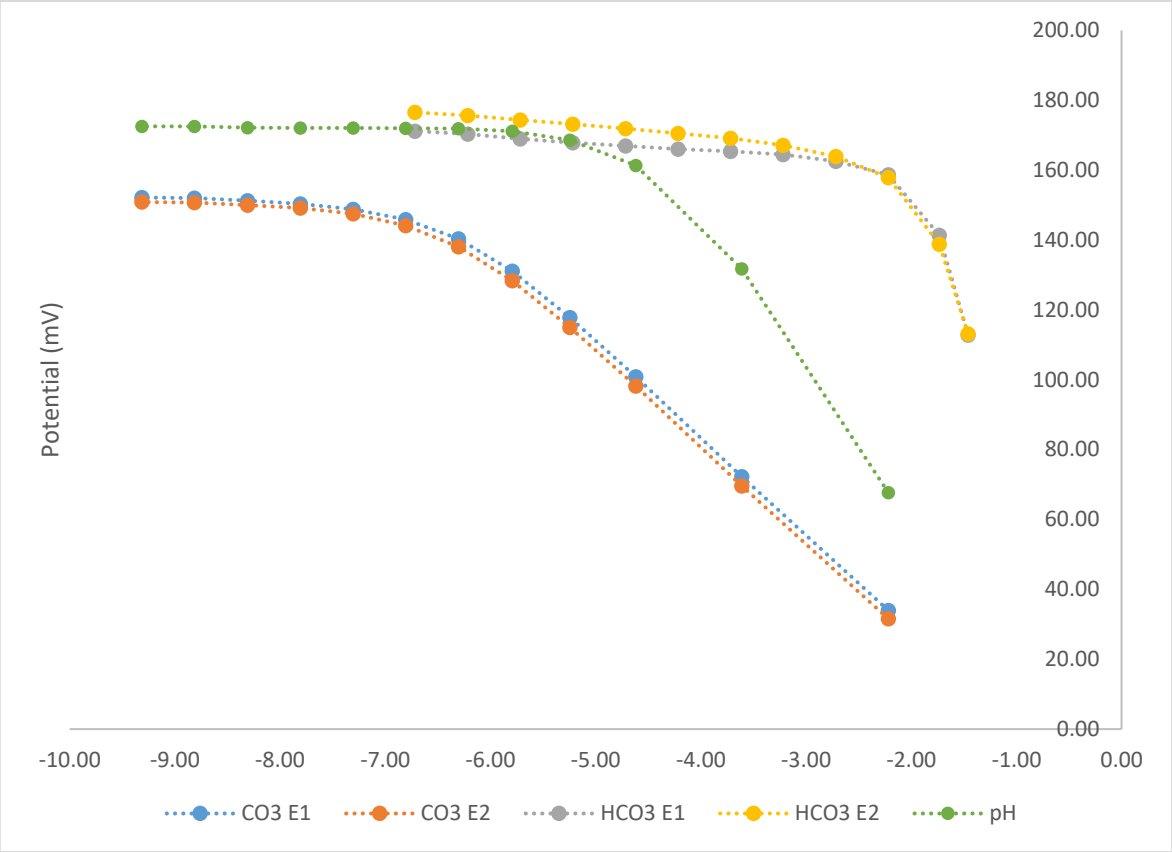

Figure 25: Conventional Electrodes in Carbonate (Tris Buffer ~8.6 pH)

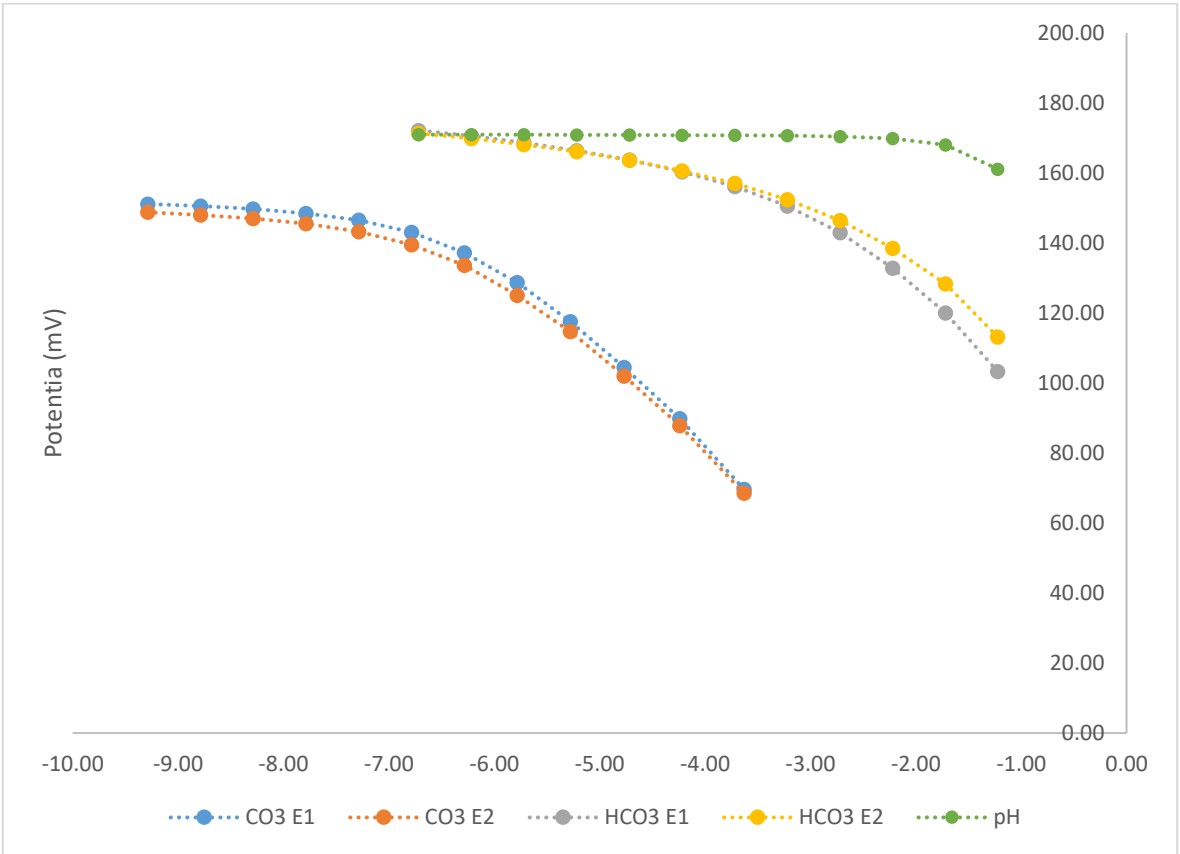

Figure 26: Conventional Electrodes in Bicarbonate (Tris Buffer ~8.6 pH)

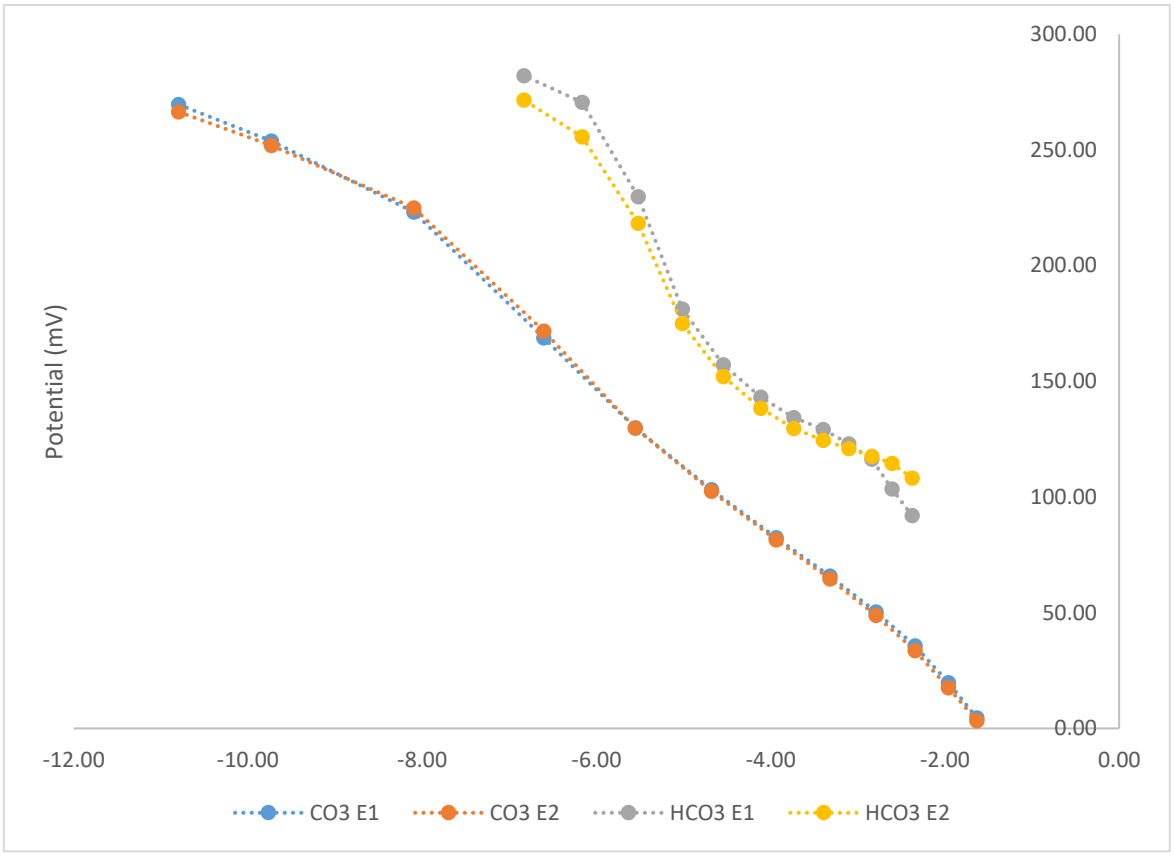

Figure 27: Conventional Electrodes in Carbonate (Boiled Water)

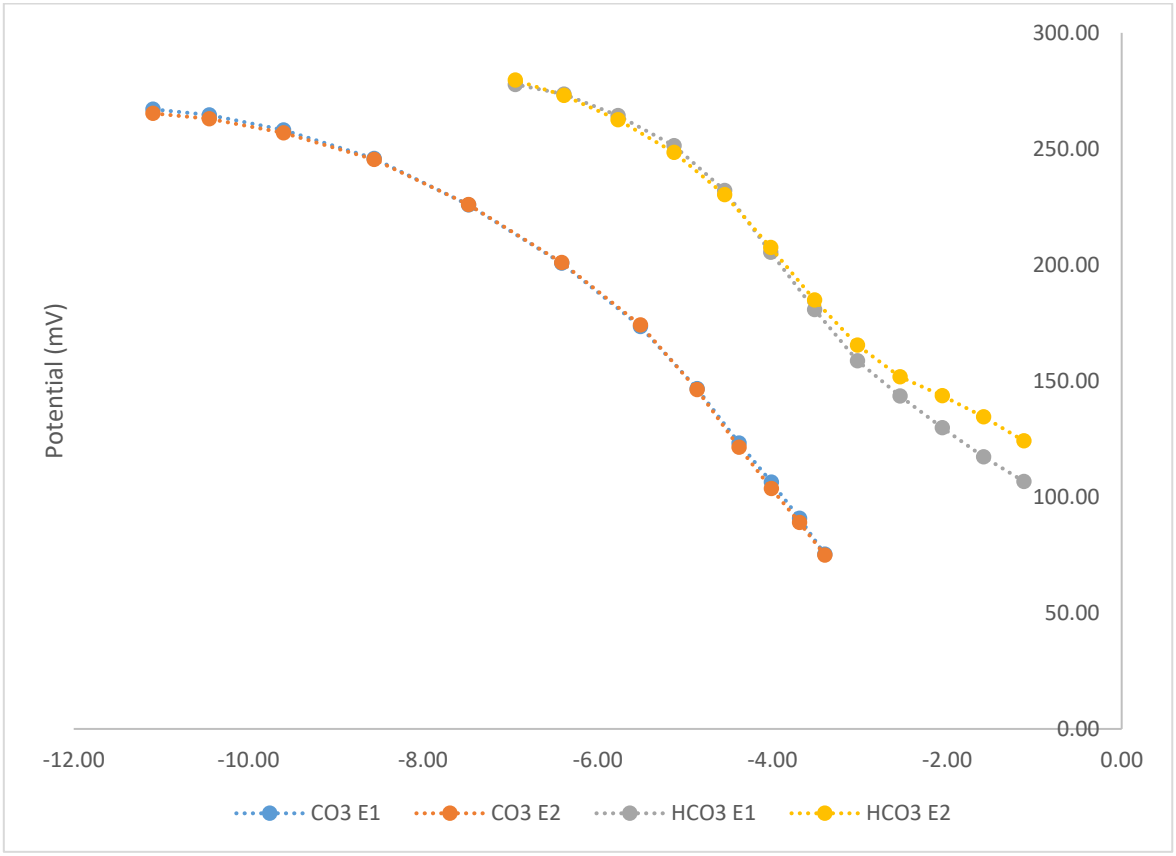

Figure 28: Conventional Electrodes in Bicarbonate (Boiled Water)

Appendix C: Derivation of Equations

Derivation of fourth order polynomial for predicting the pH. Following shorthand notations are used for clarity.

$A = [H^+]$, $B = [HCO_3^-]$, $C = [H_2CO_3]$, $D = [CO_3^{2-}]$, $E = [OH^-]$, $N = [Na^+]$, $C_T = [DIC]$

Equilibria,

$$K_1 = \frac{AB}{C}$$

$$K_2 = \frac{AD}{B}$$

$$K_W = AE$$

Mass Balance,

$$C_T = C + B + D$$

Charge Balance,

$$0 = N + A - B - 2D - E$$

$$B = \frac{K_1 C}{A}$$

$$B = \frac{AD}{K_2}$$

$$E = \frac{K_W}{A}$$

$$C = \frac{AB}{K_1} = \frac{A^2 D}{K_1 K_2}$$

$$D = C_T - C - B = C_T - \frac{A^2 D}{K_1 K_2} - \frac{AD}{K_2}$$

$$D = C_T - \frac{A^2 D + K_1 AD}{K_1 K_2}$$

$$C_T = D + \frac{A^2D + K_1AD}{K_1K_2}$$

$$C_T = \frac{D(K_1K_2 + A^2 + K_1A)}{K_1K_2}$$

$$D = \frac{C_TK_1K_2}{(K_1K_2 + A^2 + K_1A)}$$

$$B = \frac{AD}{K_2} = \frac{A(C_TK_1K_2)}{K_2(K_1K_2 + A^2 + K_1A)}$$

$$0 = N + A - \frac{A(C_TK_1K_2)}{K_2(K_1K_2 + A^2 + K_1A)} - \frac{2C_TK_1K_2}{(K_1K_2 + A^2 + K_1A)} - \frac{K_W}{A}$$

$$0 = N + A - \frac{AC_TK_1K_2 + 2C_TK_1{K_2}^2}{K_2(K_1K_2 + A^2 + K_1A)} - \frac{K_W}{A}$$

$$0 = N + A - \frac{A(AC_TK_1 + 2C_TK_1K_2) + K_W(K_1K_2 + A^2 + K_1A)}{A(K_1K_2 + A^2 + K_1A)}$$

$$0 = (N + A)A(K_1K_2 + A^2 + K_1A) - A(AC_TK_1 + 2C_TK_1K_2) - K_W(K_1K_2 + A^2 + K_1A)$$

$$0 = K_1K_2(NA + A^2) + A^2(NA + A^2) + K_1A(NA + A^2) - A^2C_TK_1 - 2AC_TK_1K_2 - K_WK_1K_2 - K_WA^2 - K_WK_1A$$

$$0 = K_1K_2NA + K_1K_2A^2 + NA^3 + A^4 + K_1NA^2 + K_1A^3 - A^2C_TK_1 - 2AC_TK_1K_2 - K_WK_1K_2 - K_WA^2 - K_WK_1A$$

$$A^4 + (N + K_1)A^3 + (K_1K_2 + K_1N - C_TK_1 - K_W)A^2 + (K_1K_2N - 2C_TK_1K_2 - K_WK_1)A - K_WK_1K_2 = 0$$

Therefore,

$$[H^+]^4 + ([Na^+] + K_1)[H^+]^3 + (K_1K_2 + K_1[Na^+] - C_TK_1 - K_W)[H^+]^2 + (K_1K_2[Na^+] - 2C_TK_1K_2 - K_WK_1)[H^+] - K_WK_1K_2 = 0$$

In the case of $NaHCO_3$, N = $[Na^+]$ = C_T and for Na_2CO_3, N = $[Na^+]$ = $2C_T$.